Experimental Ecological Genetics of Fragmented Populations of the Common Plant *Lychnis flos-cuculi* L.

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S Totemügerli

Chapter 1

General introduction

Biodiversity has been highly impaired during the last century. Lande even stated that "If there are any palaeontologists in the distant future, our modern age —the 20th and 21st centuries— will likely be recorded as a period of one of the greatest mass extinctions of all time, comparable to the event 65 million years ago in which it can be estimated that the majority of species then living on Earth perished" (Lande 1988). One of the major threats for biodiversity is habitat fragmentation (Soulé 1980) and more recently it has been designed as part of the "evil quartet", together with over-harvesting, exotic species, and chain of extinction (Stockwell *et al.* 2003).

Habitat fragmentation affects biodiversity at all levels: ecosystem, species and genetic. The last one, genetic diversity, had already been pointed it out in the 70s (Frankel 1974) but became an important issue only later and genetic conservation is nowadays a field of study in itself (Hedrick 2004). In the context of habitat fragmentation, genetic diversity plays an important part also because of its role in the persistence and survival of many plant populations and enables them to adapt to their changing local conditions.

This thesis addresses the effects of habitat fragmentation on the common but already declining *Lychnis flos-cuculi*, using highly integrative approaches. It is part of a longer, 6 year project (Galeuchet 2003; Perret 2003; Hoehn 2006) which allow a broader look on the subject. I assess the combined effects of plasticity, local adaptation, genetic diversity, population size and ecological conditions on plant performance. In this first chapter I introduce the general scientific background of habitat fragmentation and its actual state of research, present more specifically the adaptive and non-adaptive influences habitat fragmentation exerts onto plant populations and their consequences for populations and plant fitness. I also present the four main questions I aim to answer in this thesis and outline its contents.

CONTEXT

Habitat fragmentation

Since the seminal paper of Soulé (1980), a lot of attention has been given to the question of habitat fragmentation and several books and reviews have dealt with its effects on populations (Young *et al.* 1996; Debinski 2000; Fahrig 2003; Lienert 2004; Frankham 2005; Reed 2005). Habitat fragmentation decreases population size and increases isolation and is a threat for both rare and common species. Therefore we need to understand the demography and genetics of small populations but also the ecology and evolution of abundant species (Lande & Shannon 1996). Indeed the rare species, consisting of only small populations, are close to the end point of the habitat fragmentation effects. To evaluate the processes induced by habitat fragmentation, it is important to use populations that only recently experienced changes in their habitat and are responding to it. Moreover, abundant species may be more important for the overall functioning of the ecosystems than rare species.

Extinction

Habitat fragmentation increases population extinction risks and smaller populations were reported to have higher risks of extinction (Fischer & Stocklin 1997; Matthies *et al.* 2004). Population may enter a so-called extinction vortex, where the negative influences of genetic, demographic and environmental factors lead to always decreasing plant fitness.

Species richness is decreasing in fragmented habitats (Cousins 2006; Helm *et al.* 2006) and many local populations have gone extinct (Fischer & Stocklin 1997; Fischer 2000). Characteristics to predict which species would be most likely to go extinct have found that rare, isolated (Fischer & Stocklin 1997) and predaceous species were most at risks (Davies *et al.* 2000). Simulations have found that a threshold of remnant habitat smaller than 10 to 30 % needed to be reached before the threat of habitat fragmentation on population persistence become obvious. Better knowledge of these thresholds taking into account each species

and landscape specificity should be useful in conservation and management practices (Huggett 2005). Solé *et al.* also found a threshold for overall biodiversity collapse (2004).

Adaptive processes: plasticity and local adaptation

Habitat fragmentation changes habitat quality in several ways. Light and humidity are often highly influenced by the nature of the landscape matrix surrounding the natural habitat of the population. In agricultural matrices, inputs of nutrients and pesticide become stronger and soil conditions deteriorate. Disturbances of all kinds increase in frequency. Plants have two main ways to respond to such habitat variation: either they can change their phenotype plastically or they can evolve new adaptations.

Phenotypic plasticity could partly compensate the variation in local conditions. However, the evolution of phenotypic plasticity has limits and costs (DeWitt *et al.* 1998) and the new environmental conditions can be out of reach from a possible plastic response. Moreover, it is not known if phenotypic plasticity can fully compensate for a lack of genetic variation in heterogeneous environments (Booy *et al.* 2000). Phenotypic plasticity is hard to select for in experiments (Fischer *et al.* 2004) and many factors can influence the evolution of plasticity in plants (Kingsolver *et al.* 2002).

Local adaptation enhances plant performances (Joshi *et al.* 2001) and is genetically based (Latta *et al.* 2004). A recent review has dealt with local adaptation and its characteristics (Kawecki & Ebert 2004). However, the existing local adaptation might slow further adaptation to changes and overall impair plant capacity to respond to new habitat characteristics. This has led to transplantation failure, and/or to lower success in conservation measures (Van Andel 1998) and to concern regarding management practice in population conservation. However, populations can adapt to the new conditions if they still retain the capacity to evolve. One important aspect would be to know how habitat fragmentation and local adaptation interact to influence plant

performances in natural populations.

Genetic erosion

Habitat fragmentation is also highly detrimental for the genetic health of populations (Ellstrand & Elam 1993). Selection could decrease genetic diversity in an adaptive way, acting to favour advantageous genotypes. However, it has far less influence when populations are small as it has only a small number of individual to act upon and other genetic mechanisms such as drift and inbreeding drive the genetic diversity in small populations. Habitat fragmentation is known to determine genetic variation in population (Gibbs 2001), bringing non-adaptive changes via drift. Loss of genetic diversity decreases population fitness on the short term and adaptation potential on the long term. Genetic variability is crucial on the long-term for persistence and survival of populations (Lande & Shannon 1996).

Rare plants have been found to have less genetic variation than common plants (Cole 2003) and genetic diversity increases with population size in many species using several measures and indices (Oostermeijer 1996; Fischer & Matthies 1998b; Fischer *et al.* 2000; Van Rossum *et al.* 2002; Tomimatsu & Ohara 2003) but see (Leimu & Mutikainen 2005).

In parallel, plants show decreased performances in small compared to large populations (Aizen & Feinsinger 1994; Heschel & Paige 1995; Oostermeijer 1996; Fischer & Matthies 1998a; Kery 2000; Hackney & McGraw 2001; Van Rossum *et al.* 2002; Aigner 2004; Kolb 2005) but see (Costin *et al.* 2001). Population genetic structure has been studied in many species at the landscape level, both with the present and past landscape characteristics (Prentice *et al.* 2006) but see (Honnay *et al.* 2006). Between populations, F_{st} has been used to evaluate how similar or different populations were. Neutral genetic variation has been the focus of most studies, but quantitative variation can also be used to estimate genetic variation (Carvajal-Rodriguez *et al.* 2005), and this may be more

important as it is the genetic part most important for population evolution and survival (Storfer 1996). A meta-analysis found that population fitness was significantly correlated with genetic diversity and it explained 19% of the variation in fitness (Reed & Frankham 2003).

Inbreeding and drift

Inbreeding depression is one of the mechanisms decreasing plant fitness in small populations. Inbreeding can be due to selfing or to biparental inbreeding (Hauser & Siegismund 2000; Griffin & Eckert 2003) and can be measured with F_{is}. Thus it increases the chance of two homologous recessive detrimental alleles to be found in an individual and to act there (Keller & Waller 2002). Inbreeding depression is mostly found in early life history stage (Hauser & Loeschcke 1995; Husband & Schemske 1996; Fischer & Matthies 1997; Emery & McCauley 2002), but also occurs in later stages (Weller *et al.* 2005) and has been found in captive and wild populations (Crnokrak & Roff 1999). Habitat fragmentation reduces not only the census population size but also effective population size Ne (Alo & Turner 2005) and this in turn increases inbreeding (Newman & Pilson 1997). A positive effect of inbreeding is that it could allow the purging of genetic load in small populations. This has not been proved to happen consistently (Byers & Waller 1999; Kirkpatrick & Jarne 2000) but a review of experimental evidence concluded that it could be more common than previously thought, but masked by the variation between study methods and between taxa (Crnokrak & Barrett 2002). Longer-term inbreeding has hardly been studied, especially in natural populations and one necessary next step would be to study inbreeding effects on the longer-term for the fitness of plant populations in the habitat fragmentation context.

Drift is the second genetic mechanism contributing to the fitness decrease of plants from small populations. Drift is the chance of losing alleles due to random sampling of the alleles from one generation to the next. This allows the fixation of alleles at random, even if they are detrimental, which creates the so-called drift load. This will then increase

inbreeding depression, as many plants will be homozygous for these recessive detrimental alleles. Consequently, fixed deleterious alleles in combination with inbreeding contribute to the genetic load of the population and lower the performances of all plants in the population (Willi *et al.* 2005). A high genetic load increases the population extinction probability in small populations (Lynch *et al.* 1995).

Biotic interaction: focus on pollination

On a higher level, a positive relationship between species diversity and genetic diversity has been reported (Vellend & Geber 2005). Low genetic diversity in plant populations also influences the diversity of the arthropod communities in the same community (Wimp *et al.* 2004; Johnson *et al.* 2006), thus population decrease of one species can bring the decrease of other interacting species in the community.

As an example of biotic interaction, pollination has received a lot of attention (Colling *et al.* 2004). Population genetic structure and gene flow between population rely on pollinators (Berge *et al.* 1998; Cresswell & Osborne 2004) but habitat fragmentation often decrease pollination (Steffan-Dewenter 1999; Cunningham 2000; Waites & Agren 2004; Ward & Johnson 2005) or affects pollinator behaviour (Goverde *et al.* 2002). This can be detrimental especially if the plants depend on a highly specialised pollinator (Ashworth *et al.* 2004). Thus, small fragmented populations can experience a limitation in pollen quantity and/or quality. This in turn can affect the diversity of deposited pollen.

Pollen diversity

Sexual selection is a strong evolutionary force in plants (Skogsmyr & Lankinen 2002) and pollen interactions play an important role in it (Walsh & Charlesworth 1992; Bernasconi 2003). Sexual selection is even given as an explanation for the evolutionary success of the angiosperms (Mulcahy 1979). Among other mechanisms, pollen diversity is important because progeny from multiple pollen donor cross can show higher vigour

(Marshall & Ellstrand 1986; Marshall *et al.* 2000; Winsor *et al.* 2000; Kalla & Ashman 2002; Davis 2004). This in turn affects population genetic structure.

Pollen diversity could reduce inbreeding (Armbruster & Rogers 2004), especially if it favours outbred compared to self pollen (Aizen *et al.* 1990). Pollen interactions can also affect gene flow between populations depending on preferences for pollen from the same or from another population (Rognli *et al.* 2000). Indeed, genetic dissimilarity can be a critical factor to gain the favours of the maternal plants and outbred pollen can then be preferred to local pollen (Souto *et al.* 2002). Other studies have shown preference for within population pollen, probably due to the local pollen being more likely to be adapted to the local pistils (Cruzan 1990; Baker & Shore 1995). Thus effects of pollen diversity can vary in the fragmented landscape and play a crucial role on how populations are able to cope with habitat fragmentation.

Pollen interaction has mostly been studied as resulting in pollen competition and selection between pollen and/or father quality. An alternative possibility would be that pollen interaction results in complementarity effects, where the combination of fathers is beneficial. Most studies also considered only one or few populations and disregarded population characteristics such as population size and genetic diversity within population. However, these are very likely to influence effects of pollen diversity in the field, as it is already known that they can influence the behaviour and efficiency of pollinators. Therefore, it is now necessary to separate complementarity and selection effects in pollen diversity experiment and to evaluate the influence of habitat fragmentation on pollen diversity effects.

Genetic rescue

To face the decrease in genetic diversity in many species, increasing gene flow between populations has been suggested to increase genetic diversity within populations (Ellstrand *et al.* 1989; Ellstrand 1992) and

reduce inbreeding (Richards 2000). Storfer argued that the past and present gene flow rates should be known before artificially enhancing it (Storfer 1999) and population history should also be included to evaluate how gene flow should be restored (Booy *et al.* 2000).

Genetic rescue can replenish lost genetic variation (Ingvarsson 2001) and therefore can be expected to have different effects depending on the population size of origin. Genetic rescue will be more important for small, genetically poor recipient populations. An heterosis effect is often present in the first generation of outbreeding (Luijten *et al.* 2002; Weller *et al.* 2005). Crosses between populations have been found to have a positive effect (Hauser & Loeschcke 1994; Bailey & McCauley 2006) and sometimes even for several generations (Edmands 1999). This has been modelled by Lynch (1991). Vilas found for *Silene littorea* that this positive effect was due to reversing inbreeding depression rather than increasing genetic diversity per se (Vilas *et al.* 2006). It could be seen as similar to recolonisation of habitat by species that had gone locally extinct (Gonzalez *et al.* 1998). However, genetic rescue is not an easy concept and entailed concerns for neutral, detrimental and adaptive variation (Tallmon *et al.* 2004; Hedrick 2005) and Stockwell even said that "Gene flow is the Jekyll and Hyde of conservation" (Stockwell *et al.* 2003). Its effects will depend a lot on both source and recipient populations' characteristics and most studies until now have dealt with too few populations (often three or four populations) to allow for broad insights on genetic rescue and its consequences.

Break down of co-adapted gene complexes and of local adaptation

Crosses between populations can also have detrimental effects. Some outbreeding depression can occur and thus be a serious cost to an increase of genetic diversity. Outbreeding depression can be link either to genetic break down or disruption of local adaptation.

Outbreeding depression was found to affect plant fitness (Price & Waser 1979; Fischer & Matthies 1998a; Sagvik *et al.* 2005) but see

(Bailey & McCauley 2006). Genetic break down linked to co-adapted gene complex has also been documented (Willett & Burton 2003). Moreover, genetic introgression can also be detrimental (Keller *et al.* 2000). We can expect different responses of plant fitness to outbreeding depending on the size of the recipient populations. Small inbred populations can show a higher co-adapted genome because of inbreeding. At the same time, local adaptation is less likely in small populations as selection is weak and drift is the major force shaping genetic diversity. There is usually an optimal outcrossing distance between populations (Waser & Price 1989) but this is however highly variable in space and time and depends on the populations involved (Colling *et al.* 2004; Bailey & McCauley 2006). Mechanisms such as epistasis (Fenster *et al.* 1997; Wade 2002) and pleiotropy also complicate the understanding of what to expect from between populations crosses.

The break down of local adaptation should be avoided (Hufford & Mazer 2003) but it often depends on which populations are available for conservation purposes. There is often a trade-off between increasing heterosis and preventing a decrease in local adaptation (Vergeer *et al.* 2004): using the most genetically diverse population possible or even a combination of populations as source material for reinforcement has to be weighted against using the most similar population. This needs to be studied with a sufficient number of populations to have the statistical power to incorporate the influence of habitat fragmentation on the outcome of crosses between populations.

AIM of this STUDY

I examined the interaction between habitat fragmentation, population genetics and local adaptation. I first asked what the consequences are of longer-term inbreeding for the fitness of *L. flos-cuculi* plants from a fragmented landscape. I tackled the interaction between habitat fragmentation and local adaptation, which had not before been simultaneously addressed, in a reciprocal replant-transplant experiment

among 15 populations. I also looked at pollen diversity and its effects on plant performances and on population via pollen competition, which is an often neglected level of diversity, and there I tried to separate selection and complementarity effects with a pollen diversity experiment, for the first time applying a method from community ecology. To finish, I focused on the effects of inter-population crosses for 13 populations of *L. flos-cuculi*, 6 small and 7 large.

I used several approaches, including greenhouse experiments, population crosses, field transplantations and molecular laboratory work. I used a still common species to tackle these issues. Indeed, the effects of habitat fragmentation are already present in such species, and the process itself can be studied, rather that the end point represented by rare species. I have also been able to manipulate large number of plants and populations and to do so without risks of extinguishing the populations, which would not be possible for rare species. The results from this thesis will allow not only to answer several new open questions in plant evolutionary ecology, but also to specify several recommendations for conservation measures.

STUDY SPECIES

I addressed the previous questions using the ragged robin *Lychnis flos-cuculi* (= *Silene flos-cuculi* (L.) Clairv., *Coronaria flos-cuculi* (L.) Braun) (Caryophyllaceae). This herb is common throughout all Europe, except for its Artic regions. It grows in sunny, wet-to-moist habitat such as fens and wet meadows and can be found from the plain to the montane level.

This rosette forming, perennial plant reproduces both sexually and clonally and produces several flowering stems 20-90cm high, bearing up to 50 flowers in dichasial inflorescence. The flowers are protandrous and the plants are mainly outcrossed (Biere 1996), but selfing occurs. The main pollinators are Diptera and Hymenoptera (*Bumbus*), and occasionally Lepidoptera (Vejsnæs & Høvsgaad 1990). In our study area, the flowering period occurs mainly in June-July while the fruits are

mature in August. The fruit capsule contain up to 200 seeds, which are dispersed around the mother plant by vibration of the stiffened stalk. The seeds are able to germinate in autumn and in spring (Biere 1991). The five stigmas present receptive hair on most of their length thus providing a large surface for depositing and spreading pollen. The pollen/ovule ratio is high, 192 $\pm$23 (Jurgens *et al.* 2002).

Although still common, its populations have been declining in the last decades due to changed in agriculture and urbanisation, and therefore it is a good model for studying the process of fragmentation. As applied aspect, this plant is often used in restoration measures in Switzerland such as extended field margins in the agricultural landscape or meadow restorations (Lehmann *et al.* 2000).


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207. Coronaria flos cuculi A. Braun. Kukuksblume.

Study sites

I chose the 8 largest (> 10'000 flowering stalks, counted at the peak of flowering, in June-July 2000) and 7 smallest populations (< 1'000) of a larger set of 28 populations used for various demographic and genetic studies (Galeuchet *et al.* 2005a, 2005b) and all experiments presented in this thesis will refer to these 15 populations or a subset of them. They were all situated in the north east of Switzerland at altitudes of 850–1350 m in fen meadows, which are mown yearly in September and are not supplied with fertilizer. Calcareous fen meadows require nutrient-poor, base-rich, hydrological input and they therefore mostly occur in depressions with a high groundwater table or on slopes with aquifer discharges (Ellenberg 1982).

These populations provided various population sizes and genetic diversities to estimate effects of habitat fragmentation and a large panel of different ecological conditions (such as moisture, light, nutrients…) to test for local adaptation, yet geographically close enough (69 km apart at most) to experience a similar overall climate and to have gene flow between them (Galeuchet *et al.* 2005a).

OUTLINE of this THESIS

In **Chapter 2**, I studied with my colleagues how inbreeding depression might differ between populations depending on population history and change over generations because of dominance and epistasis or because of selection purging deleterious mutations. Therefore, I tested the consequences of three generations of experimental selfing and within-population outcrossing for plants from 19 natural populations differing in both size and degree of isolation of the self-compatible perennial plant species *Lychnis flos-cuculi*.

In **Chapter 3**, I examined the relative importance of plastic responses to differences in habitat quality and local adaptation for plant performance in the fragmented landscape. I performed a reciprocal replant-transplant experiment among 15 populations and monitored the plants during four years. This will show the yearly variability of fitness components as well as the changing importance of various environmental and genetic factors in the establishment and development of plant populations.

In **chapter 4**, I report a greenhouse experiment on plants from 8 populations of different sizes after crosses with two levels of diversity (1 and 4 pollen donors) and microsatellites paternity analyses to evaluate effects of pollen diversity in the fragmented landscape. Moreover, I present a new analysis method to partition the effect of pollen paternity on offspring fitness into sampling and complementarity effect.

The **Chapter 5** deals with the effects of local adaptation, populations differentiation, and population sizes on the outcome of gene flow between populations. To evaluate the consequences of adaptation to landscape heterogeneity and fragmentation, I crossed 7 plants of each of 13 populations within and between populations, also taking the effects of paternal population characteristics into account. I performed F2 and backcross crosses the following year. The results of this study should allow better use of population reinforcement for threaten populations.

To conclude, I summarize the main results of this three-year project in **chapter 6**. I then draw overall conclusions and highlight some new questions lying ahead.

Chapter 2

Fitness consequences of three generations of inbreeding of *Lychnis flos-cuculi* L. plants from 19 populations from a fragmented landscape

With Sophie Hoehn, Catherine Perret,
David Galeuchet and Markus Fischer

Key words: Allee effects, inbreeding depression, landscape fragmentation, *Lychnis flos-cuculi*, purging, self-pollination.

Abstract

In the fragmented landscape, isolation of populations and small population size increase rates of inbreeding within populations. This may lower plant fitness via inbreeding depression. However, depending on population history inbreeding load may differ between populations. It can change over generations because of dominance and epistasis or because of selection against deleterious alleles. We tested the consequences of three generations of crosses of different degrees of experimental selfing and outcrossing for plants of the self-compatible perennial plant species *Lychnis flos-cuculi* from 19 populations in Northeast Switzerland. These populations of plant origin differed in size and genetic diversity. Inbreeding depression was expressed in all generations and at the stages of seed production, germination and adult plant size. Genetic load was substantial in small populations and it was not purged rapidly under selfing. While few interactions between levels of experimental inbreeding and genetic variation of the population of origin suggested purging of inbreeding load from genetically less variable natural populations, others did not. We conclude that common plants, such as *Lychnis flos-cuculi,* do not rapidly purge their inbreeding load if populations get small and are therefore prone to negative genetics consequences of landscape fragmentation.

Introduction

Nowadays, habitat fragmentation threatens the survival of many species because their populations become small and more isolated (Young et al. 1996, Stephens and Sutherland 1999, Serrano et al. 2002, Hooftman and Diemer 2003, Hooftman et al. 2004, Lienert 2004). For natural populations a reduction in size is associated with several demographic and genetic consequences including an increase of random genetic drift and an increase in inbreeding rate (Oostermeijer et al. 1994, Oostermeijer 1996, Young et al. 1996).

Allee effects are defined as mechanisms which lead to a positive relationship between individual fitness traits and the number or density of conspecifics in a population (Young et al. 1996). Some Allee effects are genetically based. One mechanism that can lead to genetic Allee effects, in combination with increased rates of inbreeding in small populations, is inbreeding depression. Inbreeding depression occurs when plant fitness is reduced by the breakdown of heterozygote advantages (overdominance in one or several loci) or by the expression of recessive deleterious alleles (Dudash 1990). The strength of inbreeding depression can differ between different stages of the plant life cycle. Expression of lethal or severely deleterious recessive alleles is supposed to happen in the earliest stages of the life-cycle resulting in seed abortion or seedling mortality, while less deleterious alleles might be accumulated and decrease plant fitness later in the life-cycle by reducing growth rate or reproduction (Lande and Schemske 1985, Dudash 1990, Hauser and Loeschcke 1994, Cheptou et al. 2000)

However, inbreeding depression does not necessary lead to Allee effects. In the long run, inbreeding load may be purged by selection against plants homozygous for detrimental alleles (Dole and Kermit 1993, Hauser and Loeschcke 1994, Young et al. 1996, Carr and Dudash 1997). In smaller populations with lower genetic variability and higher inbreeding rates (Hauser and Loeschcke 1994, Oostermeijer 1996, Byers and Waller 1999, Kephart et al.

1999, Gibbs 2001, Hooftman et al. 2004) inbreeding load might be purged more rapidly by selection than in larger populations.

Genetic Allee effects need not only be caused by inbreeding and inbreeding depression, but also by the fixation of deleterious alleles which is more likely in smaller populations. Once deleterious alleles are fixed in a population, they cannot be purged anymore by selection.

Our model plant species *Lychnis flos-cuculi* (L.) (Caryophyllaceae) is a common perennial plant of sunny and moist-to-wet meadows. In Switzerland, wetlands were reduced by 80% during the last century (Broggi and Schlegel 1989). Thus, *Lychnis flos-cuculi* has suffered from habitat destruction and fragmentation for several decades. A microsatellite study revealed that levels of within population inbreeding, F_{is}, are high (Galeuchet et al. 2005a) and genetic variation is reduced in smaller populations of *Lychnis flos-cuculi*. Moreover, plants from smaller populations perform worse in the greenhouse, indicating genetic Allee effects (Galeuchet et al. 2005b).

We focus on effects of inbreeding on the fitness of *L. flos-cuculi* plants from small and large populations. More specifically, we address the following questions: 1-How is *L. flos-cuculi* affected by inbreeding at different stages of its life cycle? 2-What are the consequences of longer-term inbreeding for *L. flos-cuculi?* 3-Are plant populations able to purge their genetic load, and if so, do smaller and more inbred populations purge their genetic load more efficiently than larger, more genetically variable populations?

Material & Methods

Study Species

Lychnis flos-cuculi L. (=*Silene flos-cuculi* (L.) Clairv. Caryophyllaceae) is a common polycarpic perennial herb of sunny and moist-to-wet meadows. It is widespread from the plains to montane elevations across most of Switzerland and Europe (Aeschimann and Burdet 1994, Delarze et al. 1998, Lauber and Wagner 2000). Traditionally the species occurred abundantly in wet hay meadows or calcareous fens but in recent decades these habitats were severely fragmented (Broggi and Schlegel 1989, Keller 1996). Therefore, the species has recently declined in number and size of populations.

During the first growing season, a primary rosette is formed and usually several secondary rosettes grow from auxiliary buds. During the second growing season, one or more rosettes can become reproductive and grow flowering stems of 30-90 cm height (Lauber and Wagner 2000). The inflorescence is a dichasium with about 20 flowers.

Flowering season in the study area is in June and July. Flowers are typically protandrous, favouring outbreeding, but geitonogamous, self-pollinations do occur. Main pollinators are bumblebees (*Bombus* sp.) and Diptera, occasionally also Lepidoptera (Noctuidea) (Vejsnæs and Høvsgaad 1990, Hauser and Loeschcke 1994).

Fruits mature within 3 to 6 weeks (Galeuchet 2003). Mature fruit capsules, which contain up to 200 seeds, open at the top and seeds are dispersed when stalks are shaken by wind or animals.

Study Populations

For this study, we selected plants of 19 wild populations. All populations were located at altitudes between 860 m and 1380 m in the North-East of Switzerland, in the Cantons St-Gallen, Schwyz, and Appenzell (Table 1). The populations were situated in fen meadows mown yearly in September and not supplied with

fertilizers. Population sizes were estimated by counting the number of flowering individuals at peak of flowering during June and July of 2000, 2001, 2003 and 2004. In 2004 estimated population sizes ranged from 30 to 63'000 flowering stems.

Table 1: Canton (AR = Appenzell Ausserrohden, SG = St-Gallen, SZ = Schwyz), Population size (average size from 2000 to 2004), altitude, Swiss geographic coordinates of the 19 *Lychnis flos-cuculi* populations studied and respective microsatellite allelic richness (AR) and inbreeding coefficient (Fis) (Galeuchet et al. 2005a).

Site	Canton	Pop size	Altitude (m)	Coordinates		Sample size	Allelic richness AR	Mean inbreeding coefficient Fis
				E	N			
Allmeindswald	SG	204	1080	732 220	237 525	15.571	5.0	0.335
Gäbris Wald	AR	218	1175	753 430	249 915	15.714	4.8	0.525
Höchi	SG	302	970	725 865	234 945	16.714	4.4	0.448
Hasenried	SG	381	1185	740 520	233 400	16.714	4.7	0.529
Haumösli 1	AR	539	940	740 935	242 245	8.286	4.7	0.452
Gäbris Seeli	AR	979	1020	753 945	249 895	13.000	4.5	0.510
Janseren	SZ	1422	930	691 700	214 785	16.143	4.4	0.416
Wäni	SZ	1604	922	699 245	218 905	15.571	4.9	0.467
Sulzel	SZ	1798	956	703 400	222 400	16.429	4.6	0.472
Chellen	SG	2740	940	730 475	237 395	17.143	5.2	0.427
Roblosen 1	SZ	3456	897	700 950	223 100	8.286	4.7	0.469
Etteren	SZ	3683	1050	697 020	213 180	10.714	5.1	0.544
Fetzeren	AR	7671	1005	735 900	241 015	16.571	5.1	0.519
Stein	SG	8562	1020	733 655	228 725	16.714	4.5	0.585
Feusisberg	SZ	8824	865	697 570	224 390	17.143	5.0	0.451
Bannholz	SZ	10917	947	704 130	221 425	15.714	4.8	0.481
Landscheidi	AR	11325	935	734 638	240 576	15.714	4.8	0.316
Feldmoos	SG	14739	934	731 175	232 095	16.571	4.9	0.521
Munzenriet	SG	43397	1185	744 845	229 390	16.714	4.8	0.403

Microsatellite variation

For each population, estimates of Allelic richness, AR, and inbreeding coefficient, F_{is}, were available from a study of 18 plants per population with seven polymorphic microsatellite loci (Galeuchet *et al*., 2005). Within-population inbreeding coefficient F_{is} varied from 0.316 to 0.585 with an average of 0.467, indicating a mixed mating system with substantial inbreeding in *L. flos-cuculi*. Allelic richness, A_R, varied between 4.4 and 5.2 with an average of 4.8 over all populations (Table 1) and increased with increasing population size.

Experimental design

Hand pollinations and seed measurements

In June 2000, we randomly selected twelve flowering *L. flos-cuculi* plants in the 19 parental populations and defined them as parental plants. We performed the first crosses (resulting in the F1) by pollinating 228 parental plants in two different ways. On each plant we performed a geitonogamous pollination (=inbred cross) between two flowers of the same plant, and an allogamous pollination (=outbred cross) between two flowers of two different plants (5-10 m away) from the same population (Fig. 1). Pollinated flowers were protected by a nylon bag to avoid any external pollen contamination. After fruit maturation we collected mature capsules, placed them separately in paper bags and dried them at room temperature for 10 days.

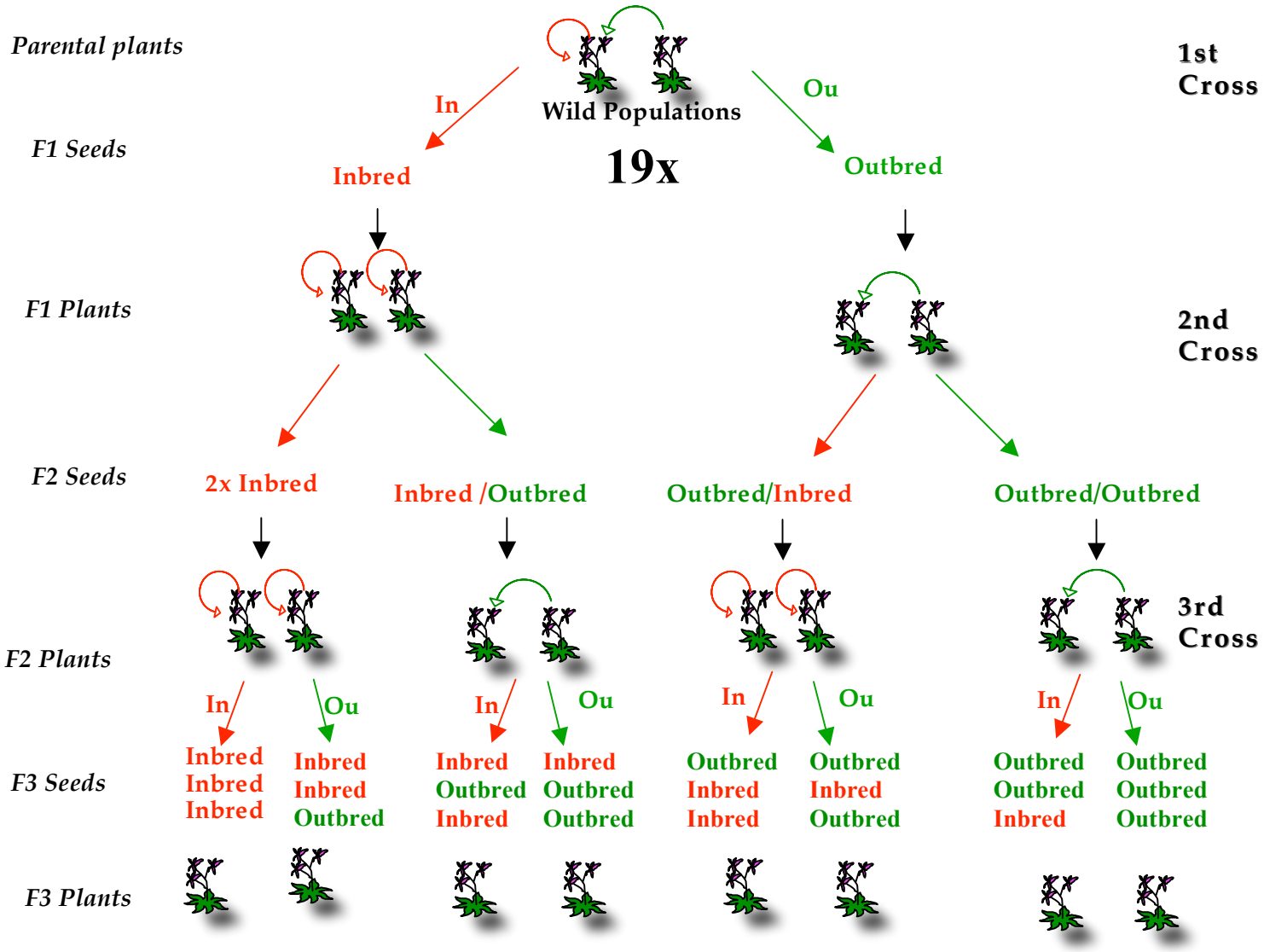

Figure 1: Design of the three-generations selfing and outcrossing experiment with plants of 19 populations of *Lychnis flos-cuculi*. Inbred crosses are crosses between two flowers of the same plant and outbred crosses are crosses between two flowers of different plants belonging to the same population.

We then counted the number of fully developed seeds (F1 seeds). We considered brown-black and round seeds as 'developed' and orange and square-edged smooth seeds as 'not developed'. We weighed all developed seeds in bulk for each capsule, and calculated individual seed mass dividing by the number of developed seeds.

In December 2000, we sowed up to 30 developed seeds per capsule in three batches of 10 seeds per 3.5 x 3.5 x 4.5 cm in multipots filled with BF4 substrate (Tref de Baat®, Coevorden, The Netherlands). We estimated germination percentage for each fruit five weeks after sowing.

In January 2001, we randomly selected two seedlings per family and transplanted them into individual 9 x 9 x 9 cm pots filled with the same substrate. We measured plant fitness (F1 plants; see next section for fitness measures) in July 2001, after most plants produced fruits.

Between March and April 2003 we carried out the second round of crosses (F2). We performed one inbred cross and one outbred cross per F1 plant, and in total pollinated 326 F1 plants. We applied the same procedure as described above for collecting (June and July 2003), counting and weighing F2 fruits and F2 seeds. We measured adult plant fitness (F1 adult plants) in September 2003.

In January 2004, we sowed F2 seeds, again following the same procedure as for the previous generation, and measured germination percentage for the F2 seeds.

Between March and April 2004 we performed the third generation of crosses (F3). We repeated the same design to obtain the third round of plants (F3). We performed one inbred cross and one outbred cross per F2 plant, and in total pollinated 560 F2 plants. We applied the same procedure as described above for collecting (June and July 2004), counting and weighing F3 capsules and F3 seeds. In September 2004 we measured adult plant fitness (F2 adult plants).

In January 2005, we sowed F3 seeds, and recorded germination percentage after five weeks. In September 2005, after fruit maturation, we measured adult

plant fitness (326 F3 plants). Finally, for each of the 19 populations we had obtained three different generations of plants, belonging to eight different treatment lines resulting from three successive crosses on the parental, F1, and F2 plants.

To assess inbreeding levels of plants in our different treatment lines we considered that one generation of self-pollination sets the inbreeding coefficient f to 0.5 for the offspring. After two and three consecutive self-pollinations f reaches a value of 0.75 and 0.875, respectively, according to the formula $f_{(g+1)} = [(1+f_{(g)})/2]$, where g denotes a generation (Maynard Smith 1989).

Adult Fitness measurements

We assessed plant fitness considering vegetative and reproductive components. As vegetative measures we counted the number of vegetative rosettes. To evaluate reproductive fitness we recorded whether plants were flowering, and the number of flowering stems and of flowers per plant.

Cumulative fitness

To assess offspring plant fitness for the whole life cycle we calculated cumulative fitness. As plant survival was 100%, we multiplied the number of fully developed seeds produced per fruit, germination percentage, and the number of flowers per plant per treatment and per generation for each of the original maternal plants:

Ψ_{F1} = Number of Seeds * Germination percentage * Number of Flowers

Ψ_{F2} = Number of Seeds * Germination percentage * Number of Flowers

Ψ_{F3} = Number of Seeds * Germination percentage * Number of Flowers

Similarly, to assess offspring fitness over the three generations we calculated total cumulative fitness for each treatment line and for each of the original maternal plants as:

$\Psi_{Tot} = \Psi_{F1} * \Psi_{F2} * \Psi_{F3}$

Genetic Load

We used cumulative fitness to estimate genetic load. For each population we obtained genetic load as the negative slope of the regression line between parental inbreeding coefficient (f) and log-transformed offspring fitness (Willi et al. 2005). In two cases, for which the estimate was slightly negative, we set it to 0.

Data Analysis

We analysed the fitness effects of characteristics of the populations of origin, of treatments, and of their interactions with hierarchical analysis of covariance (ANCOVA, type I sum of squares) with the statistical package S-PLUS (Versions 6.1/6.2, 2002 Insightful Corp.). We used stepwise model selection to remove non-significant covariates from the model. We used experimental inbreeding coefficients f as a covariate in our model. Because it is not guaranteed that one generation of experimental outbred crosses completely resets f to 0, we only considered lines that ended with uninterrupted lines of inbreeding (3x inbred, out and 2x inbred, 2x outbred and 1x inbred; Fig. 1) and the control of three subsequent outcrosses. We entered these treatment lines as factor "treatment" in the model. We tested allelic richness and inbreeding coefficient of populations of origin against remaining variation among populations, and population, f, and treatment, against the population x treatment interaction (Table 2).

Table 2: Model of hierarchical analysis of covariance (ANCOVA) for assessing effects of habitat fragmentation, crossing treatments, and their interactions on *Lychnis flos-cuculi* offspring fitness. For F1 analyses, Inbreeding and Treatment are identical. For cumulative fitness analyses residual variation equals the population x treatment interaction.

	Offspring fitness	
Source of variation	**Mean squares**	**Variance ratios (F values)**
Population size	$ms_{Pop\ size}$	$ms_{Pop\ size} / ms_{Pop}$
Allelic richness (AR)	ms_{AR}	ms_{AR} / ms_{Pop}
Inbreeding coefficient (Fis)	ms_{Fis}	ms_{Fis} / ms_{Pop}
Population	ms_{Pop}	$ms_{Pop} / ms_{Pop\ x\ treatm}$
Experimental inbreeding coefficient f	ms_{Inb}	$ms_{Inb} / ms_{Pop\ x\ treatm}$
Treatment	ms_{Treatm}	$ms_{Treatm} / ms_{Pop\ x\ treatm}$
Population size x Inbreeding	$ms_{Pop\ size\ x\ Inb}$	$ms_{Pop\ size\ x\ Inb} / ms_{Pop\ x\ treatm}$
AR x Inbreeding	$ms_{AR\ x\ Inb}$	$ms_{AR\ x\ Inb} / ms_{Pop\ x\ treatm}$
Fis x Inbreeding	$ms_{Fis\ x\ Inb}$	$ms_{Fis\ x\ Inb} / ms_{Pop\ x\ treatm}$
Population size x treatment	$ms_{Pop\ size\ x\ treatm}$	$ms_{Pop\ size\ x\ treatm} / ms_{Pop\ x\ treatm}$
AR x treatment	$ms_{AR\ x\ treatm}$	$ms_{AR\ x\ treatm} / ms_{Pop\ x\ treatm}$
Fis x treatment	$ms_{Fis\ x\ treatm}$	$ms_{Fis\ x\ treatm} / ms_{Pop\ x\ treatm}$
Population x treatment	$ms_{Pop\ x\ treatm}$	$ms_{Pop\ x\ treatm} / ms_{Residuals}$
Residuals (=Fruits)	$ms_{Residuals}$	

Results

Generation F1

Plants from populations with higher allelic richness and lower inbreeding coefficient F_{is} produced more F1 seeds regardless of the inbreeding treatment. Consequently, cumulative fitness of F1 plants was higher for populations of higher allelic richness and lower inbreeding coefficient F_{is} (Fig. 2, Appendix 1A).

The percentage of germination of seeds resulting from inbred crosses was 10.5 % lower than the one of seeds from outbred crosses (Fig. 3)

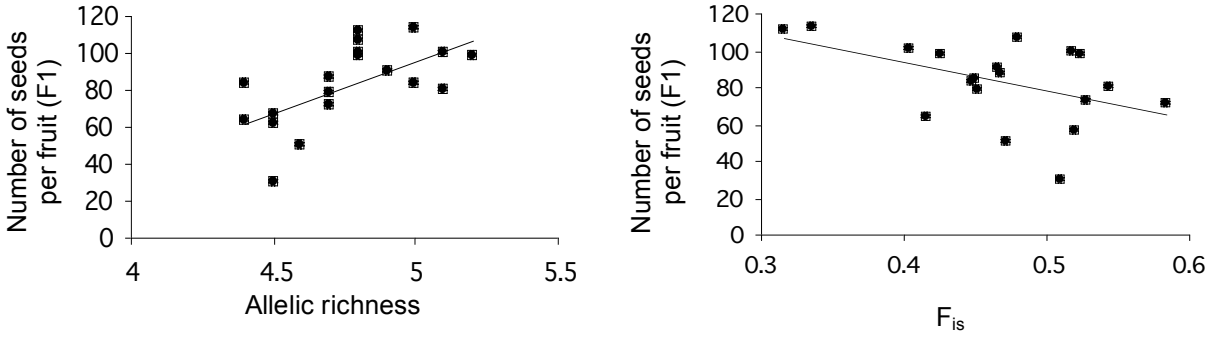

Figure 2: Influence of allelic richness and inbreeding coefficient F_{is} of populations of origin on the number of developed seeds per fruit in the F1 generation of *Lychnis flos-cuculi* (ANCOVA, $p<0.05$).

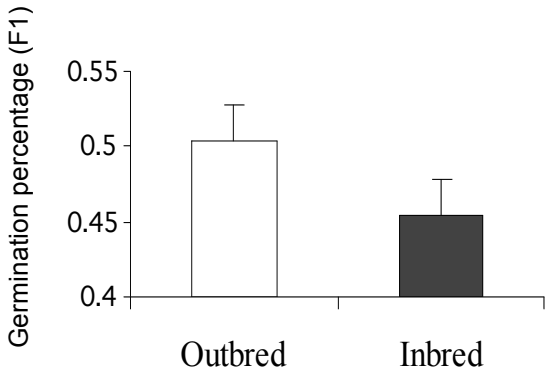

Figure 3: Effects of experimental inbreeding on germination percentage of F1 seeds of *Lychnis flos-cuculi* (ANCOVA, $p<0.01$).

Generation F2

After experimental inbreeding, inbreeding depression in cumulative fitness was stronger for plants from smaller than from larger populations of origin (Fig. 4).

F2 seeds were not affected by characteristics of the population of origin anymore. The main effect of experimental inbreeding was significant for F2

cumulative fitness (Fig. 5A; Appendix 1B) and we observed a reduction of cumulative fitness of 20.43% and of 58.64% after one and two experimental selfings, respectively. However, fruits originating from more homozygous populations had more developed seeds when at least one outbred cross had been performed, i.e. when f was 0 or 0.5 ($p<0.05$; Fig. 5B).

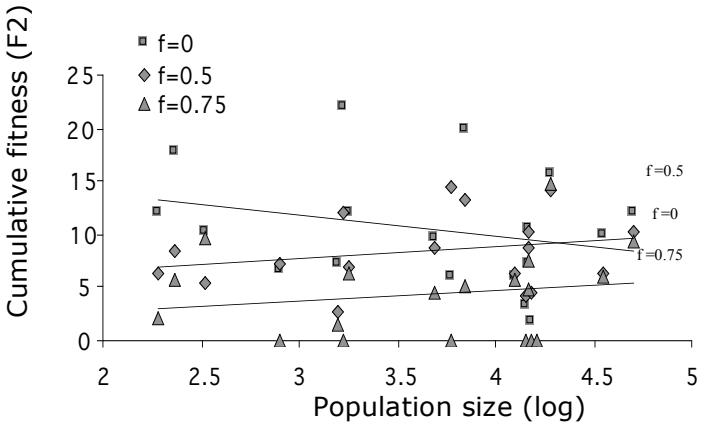

Figure 4: Larger populations of *Lychnis flos-cuculi* showed less variation in cumulative F2 fitness with varying degree of experimental inbreeding. Outbred crosses were more beneficial for F2 fitness of plants originating from smaller populations (ANCOVA, $p < 0.05$).

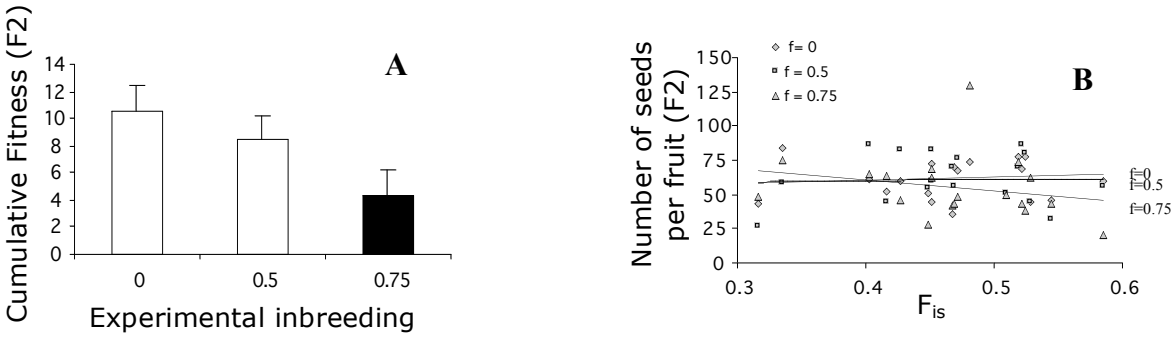

Figure 5: A) Inbreeding decreased F2 cumulative fitness after two generations of experimental selfing of *Lychnis flos-cuculi* plants (ANCOVA, $p<0.01$). B) Two generations of experimental selfing increased F2 seed production for plants originating from less inbred populations and reduced it for plants from more inbred populations.

Generation F3

Experimental inbreeding significantly reduced F3 seed germination percentage (Appendix 1C; $p<0.05$). Moreover, F3 seeds originating from larger populations had a higher germination percentage (Fig. 6). More inbred offspring lines from populations with lower allelic richness tended to have increased cumulative

fitness, as expected when inbreeding load is purged (Allelic richness x inbreeding; Appendix 1C; $p<0.1$; Fig. 7).

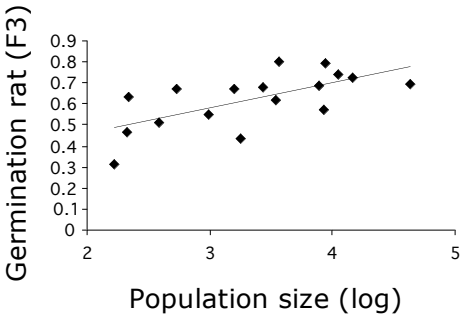

Figure 6: Plants from larger populations of *Lychnis flos-cuculi* produced F3 seeds with higher germination percentage regardless of the experimental crossing treatment (ANCOVA, $p < 0.01$).

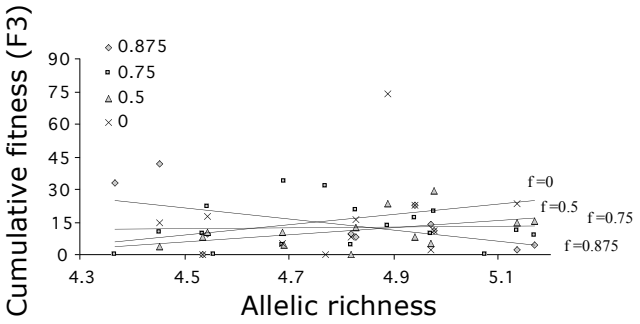

Figure 7: Effects of the interaction between inbreeding treatment and allelic richness of *Lychnis flos-cuculi* populations on F3 cumulative fitness (ANCOVA, $p<0.1$). Inbreeding depression was reduced for more inbred lines originating from less variable populations, suggesting purging of their genetic load.

Cumulative fitness over all generations

Finally, over all three generations cumulative fitness differed between populations of origin and decreased with increased experimental inbreeding (Fig. 8; $p<0.05$). Genetic load of the populations ranged from 0 to 2.28. It was independent of F_{is} and size of populations of origin, but significantly increased with increasing allelic richness of the population of origin ($p=0.05$; Fig. 9).

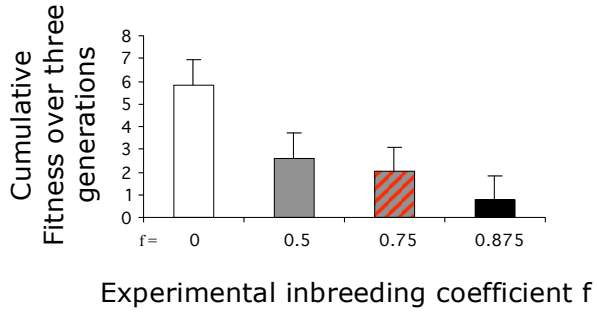

Figure 8: Cumulative fitness over three generations decreased with increasing experimental inbreeding coefficient f in the experiment with plants of *Lychnis flos-cuculi* (ANCOVA, $p<0.05$).

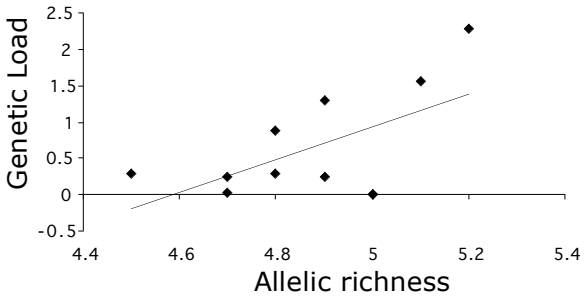

Figure 9: Genetic load was significantly reduced in populations with lower allelic richness. It suggests a reduction in inbreeding depression in smaller populations of *Lychnis flos-cuculi* due to purging (Linear regression, $p=0.05$).

Discussion

Allee effects

Our experimental conditions in the greenhouse allowed us to show that the observed differences between plants originating from different populations were due to genetic, and not due to environmental, differences. These differences in plant fitness cannot be explained by the presence of maternal carry-over effects (Roach and Wulff 1987, Oostermeijer et al. 1994, Schmid and Dolt 1994) because we did not observe any correlations between seed mass and germination or seedling mortality (Oostermeijer et al. 1994, Fischer et al. 2000). Moreover, maternal effects are usually expressed during the first stages of the life-cycle (Roach and Wulff 1987). The poorer performance of plants from smaller populations remained apparent in the F1.

We detected reduced fitness of plants originating from smaller populations. Number of F1 seeds and F1 cumulative fitness were lower with lower allelic richness of the population of origin and F3 seedlings from smaller populations had a lower germination percentage. Moreover, F1 seed production and plant fitness were reduced with higher inbreeding coefficient F_{is} of the population of origin. These results extend earlier reports of Allee effects (e.g., Fischer et al. 2000, Hackney and McGraw 2001, Galeuchet et al. 2005b, Reed 2005), because they were obtained across several experimental crossing treatments, and maintained for three generations. These results also confirm that we really did our inbreeding study with plants representing the relevant genetic background.

Inbreeding depression

Fitness of *L. flos-cuculi* plants decreased with increasing degree of experimental inbreeding. Inbreeding depression was not expressed similarly in all stages of the life cycle and some fitness components were only affected after two generations of inbreeding. Corresponding to the results of Hauser and Loeschcke (1995), we found that germination was affected after one generation of

experimental self-pollination. Contrasting with the previous results, seed production was affected by inbreeding only when two successive inbred crosses were performed.

Our results indicate that the still common species *L. flos-cuculi* suffers from inbreeding depression after one or several generations of inbreeding and depending on generation, different fitness traits are affected, which might greatly threaten population viability in a fragmented landscape.

Purging

The reduction of plant cumulative F2 fitness by inbreeding was stronger for plants from smaller populations, and inbreeding depression was also stronger for F2 plants from populations with higher F_{is}. These patterns are the opposite of the one expected if purging of inbreeding load had played a role, because there, inbreeding depression should have been reduced in small inbred populations (Hauser and Loeschcke 1994, Carr and Dudash 1997, Byers and Waller 1999). Thus, our results suggest that two generations of inbreeding are not sufficient for *Lychnis flos-cuculi* to purge the inbreeding load of smaller, less genetically variable populations.

In contrast, cumulative F3 fitness was affected by the interaction between allelic richness and experimental inbreeding in a way that less genetically variable populations had reduced inbreeding depression. Moreover, genetic load over all three generations was reduced for populations of lower allelic richness. These latter results suggest that some purging of inbreeding load is nevertheless taking place after three generations of inbreeding.

CONCLUSION

We conclude that inbreeding depression largely affects the still common *Lychnis flos-cuculi.* Inbreeding depression reduced offspring fitness at all stages of the life cycle. Moreover, inbreeding load is not rapidly purged from small

inbred populations. Consequently, plant fitness is reduced for plants in such populations. To counteract effects of inbreeding depression on small and isolated populations of *Lychnis flos-cuculi,* population sizes need to be increased, and gene flow between populations promoted, at least as far as outbreeding depression does not become a problem.

ACKNOWLEDGMENTS

We thank Catherine Perret and David Galeuchet for their work with the first cross generation, the owners and tenants of our field sites for allowing us to access these agriculturally used study sites, and Bianca and Gustav Ehrle for skilful care for our plants in the glasshouse. This research was supported by the Swiss National Science Foundation (Grants no. 31-56809.99 and 31-67876.02 to MF).

Appendix 1A

ANCOVA results for effects of population characteristics and experimental inbreeding on number and germination percentage of F1 seeds and cumulative F1 fitness of *Lychnis flos-cuculi* offspring. We present degrees of freedom (df), mean squares (ms), F, and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

Generation F1

	Number of seeds				Germination rate				Cumulative Fitness			
Source of variation	**df**	**ms**	**F**	**P**	**df**	**Ms**	**F**	**P**	**df**	**ms**	**F**	**P**
Population size	1	236.85	0.0517	0.823	1	0.0221	0.1677	0.688	1	479'605	0.4300	0.522
Allelic richness (AR)	1	33675.58	7.3551	0.015*	1	0.2100	1.5952	0.226	1	5'816'840	5.2155	0.037*
Inbreeding coefficient (Fis)	1	25588.9	5.5889	0.031*	1	0.2637	2.0031	0.177	1	6'459'239	5.7915	0.029*
Population	16	4578.56	2.1413	0.007**	15	0.1316	4.3362	3.61e-07***	15	1'115'292	0.9725	0.521
Experimental inbreeding coefficient f	1	11398.53	1.5459	0.233	1	0.1699	9.0637	0.009**	1	1'279'769	1.1159	0.308
Population size x inbreeding coeff. f	1	1998.65	0.2711	0.61	1	0.0126	0.6715	0.425	1	85'212	0.0743	0.789
AR x inbreeding coeff. f	1	2731.21	0.3704	0.552	1	0.0001	0.0044	0.948	1	197'493	0.1722	0.684
Fis x inbreeding coeff. f	1	8699.58	1.1798	0.295	1	0.0065	0.3456	0.565	1	2'026'864	1.7673	0.204
Population x treatment	15	7373.56	3.4485	2.16e-05***	15	0.0187	0.6177	0.859				
Residuals (=Fruits)	270	2138.2			241	0.0304			15	1'146'870		

Appendix 1B

ANCOVA results for F2 effects of population characteristics and experimental inbreeding on number and germination percentage of F2 seeds, and cumulative F2 fitness of *Lychnis flos-cuculi* offspring. We present degrees of freedom (df), mean squares (ms), F, and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

Generation F2

	Number of seeds				**Germination rate**				**Cumulative Fitness**			
Source of variation	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**
Population size	1	1503.06	0.390	0.542	1	0.002	0.017	0.898	1	554	0.001	0.970
Allelic richness (AR)	1	11140.77	2.889	0.110	1	0.055	0.524	0.480	1	878813	2.356	0.146
Inbreeding coefficient (Fis)	1	393.98	0.102	0.754	1	0.005	0.049	0.827	1	76220	0.204	0.658
Population	15	3856.93	2.054	0.011*	15	0.105	1.495	0.104	15	373061	3.567	0.002**
Experimental inbreeding coefficient f	1	4686.65	3.325	0.079	1	0.058	0.697	0.411	1	2889365	27.63	1.38e-05***
Treatment	1	6939.38	4.923	0.035*	1	0.052	0.618	0.438	1	379031	3.624	0.067
Population size x inbreeding coeff. f	1	159.38	0.113	0.739	1	0.035	0.421	0.522	1	560474	5.359	0.028*
AR x inbreeding coeff. f	1	261.78	0.186	0.670	1	0.007	0.085	0.773	1	87232	0.834	0.369
Fis x inbreeding coeff. f	1	1583.41	1.123	0.298	1	0.108	1.292	0.265	1	169499	1.620	0.213
Population size x treatment	1	666.38	0.473	0.497	1	0.000	0.002	0.968	1	50274	0.481	0.494
AR x treatment	1	8.08	0.006	0.940	1	0.046	0.553	0.463	1	93818	0.897	0.352
Fis x treatment	1	5726.46	4.063	0.054*	1	0.033	0.393	0.536	1	233710	2.235	0.146
Population x treatment	28	1409.46	0.751	0.819	28	0.084	1.188	0.238				
Residuals (=Fruits)	384	1877.69			355	0.070			28	104576		

Appendix 1C

ANCOVA results for effects of population characteristics and experimental inbreeding on number and germination percentage of F3 seeds, and cumulative F3 fitness of *Lychnis flos-cuculi* offspring. We present degrees of freedom (df), mean squares (ms), F, and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

Generation F3

	Number of seeds				Germination rate				Cumulative Fitness			
Source of variation	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**
Population size	1	2747.03	0.495	0.494	1	0.717	11.03	0.006**	1	1163291	0.552	0.471
Allelic richness (AR)	1	4797.94	0.864	0.369	1	0.228	3.510	0.084	1	68307	0.032	0.860
Inbreeding coefficient (Fis)	1	3.493	0.001	0.980	1	0.031	0.483	0.499	1	232728	0.110	0.745
Population	13	5550.45	2.641	0.003*	13	0.065	1.693	0.070	13	2108048	1.354	0.251
Experimental inbreeding coefficient f	1	140.84	0.073	0.789	1	0.200	6.736	0.016*	1	134035	0.086	0.772
Treatment	2	3325.7	1.732	0.197	2	0.093	3.122	0.062+	2	1254514	0.806	0.458
Popsize x inbreeding coeff. f	1	122.41	0.063	0.802	1	0.060	2.034	0.166	1	1357237	0.872	0.360
AR x inbreeding coeff. f	1	116.38	0.061	0.808	1	0.006	0.191	0.666	1	5476092	3.518	0.073+
Fis x inbreeding coeff. f	1	5.198	0.002	0.959	1	0.066	2.213	0.149	1	5017845	3.223	0.085
Population size x treatment	2	123.34	0.064	0.938	2	0.029	0.963	0.395	2	118467	0.076	0.927
AR x treatment	2	247.1	0.129	0.880	2	0.093	3.137	0.061+	2	1066284	0.685	0.514
Fis x treatment	2	9166.86	4.774	0.018*	2	0.030	1.024	0.374	2	1729169	1.111	0.346
Population x treatment	25	1920.18	0.914	0.586	25	0.030	0.773	0.769				
Residuals (=Fruits)	124	2101.58			126	0.038			24			

CHAPTER 3

Habitat fragmentation and local adaptation: a reciprocal replant-transplant experiment among 15 populations of *Lychnis flos-cuculi* L.

With Catherine Perret, Sophie Hoehn,
David Galeuchet and Markus Fischer

Key Words

Allee effect, common species, local adaptation, *Lychnis flos-cuculi*, phenotypic plasticity, population size, transplantation experiment

Abstract

Fragmentation and changes in habitat quality can both affect plant performance but have not previously been simultaneously addressed. The relationship between habitat quality and plant performance may be ruled by plasticity or by local adaptation. We examined the relative importance of plastic responses to differences in habitat quality and local adaptation to ecological conditions for plant performance in the fragmented landscape. We studied the common but declining perennial herb *Lychnis flos-cuculi* in fen grasslands in NE Switzerland in a reciprocal replant-transplant experiment among 15 populations during four years. At sites with high moisture and nutrient indicator values, transplanted *L. flos-cuculi* grew more rosettes thus indicating plastic responses. Plants originating from smaller populations grew fewer daughter rosettes and produced fewer flowering stems. This suggests that some populations of the common plant *L. flos-cuculi* have decreased to a size where they suffer from a genetically based Allee effect. Plants originating from populations with a light indicator value different from the one at the transplantation site had reduced survival and growth at the transplantation site, suggesting adaptation to an ecological gradient. Our results indicate that both habitat fragmentation, through reduced population size, and adaptation to ecological gradients contribute to the performance of *L. flos-cuculi* in modern landscapes. We conclude that habitats should be protected in adequate sizes even for common plant species. Our study also call for caution in selecting adequate plant material in ecological restoration or compensation projects to avoid break-up of population differentiation and local adaptation to ecological gradients.

INTRODUCTION

Due to the deterioration and fragmentation of their habitats, many formerly common species have recently been forced into small and isolated populations (Saunders et al. 1991; Jennersten et al. 1992; Hooftman 2001; Lienert et al. 2002; Hooftman et al. 2003). On the one hand habitats are characterized by their spatial distribution in the landscape, and on the other by environmental conditions and resource availability. Plant performance in populations of different habitat quality may depend on the genetic architecture of populations and on interactions of genotypes with the environment (Schlichting 1986). Maximizing fitness under the variable conditions of heterogeneous environments represents a major challenge for plants. Plant genotypes may respond plastically to different environmental conditions (Schlichting & Pigliucci 1998). Alternatively, environmental heterogeneity may lead to local adaptation of resident plant populations through natural selection (Linhart & Grant 1996).

Since habitat remnants are generally reduced in size, fragmented populations frequently have reduced numbers of individuals compared to the state before fragmentation. Population genetic theory predicts that such populations will experience genetic erosion due to random genetic drift and inbreeding (Ellstrand & Elam 1993), and that isolation may prevent the compensation of these effects by gene flow between populations (Barrett & Kohn 1991). Combined with inbreeding depression, decreased genetic variation may lead to reduced fitness of plants from smaller populations, i.e. to an Allee effect (Stephens *et al.* 1999). Moreover, small genetically depauperate populations may be less able to adapt to changing conditions (Saunders et al. 1991). Plant performance in a changing landscape is best understood when interactive effects of habitat quality and genetic variation of populations are considered.

In Switzerland, wetland habitats such as fen grasslands have undergone large-scale destruction, fragmentation, and deterioration during

the last century through land-use intensification. This includes high input of nutrients, abandonment of traditional management practices, and changes in ground water tables (Hintermann 1992). Our study species *Lychnis flos-cuculi* L. (Caryophyllaceae) is common in wet meadows. However, due to the decrease of wetland habitats, the species is declining, and its distribution is increasingly fragmented. Seeds of *L. flos-cuculi* are used in seed mixtures for restoration projects in many cantons in Switzerland.

Genetic differences between populations can be demonstrated conducting greenhouse or common garden experiments. However, such experiments do not directly address the potential adaptive nature of genetic variation (Rice & Mack 1991). Replant-transplant experiments in natural populations allow for a more realistic assessment of genetic and environmental effects on plant performances. They also consider biotic factors such as competition or herbivory, which might be important for the development of localized adaptations (Antonovics & Primack 1982). Therefore, reciprocal replant-transplant experiments have been used to assess the contributions of environmental and genetic variation and of their interaction to phenotypic variation (reviewed by (Bradshaw 1984; Venable 1984; Linhart & Grant 1996; Van Andel 1998)). Quantitative trait differences between populations have been documented for important features of plant structure and function (e.g. (Waser & Price 1985; Sork *et al.* 1993; Miller & Fowler 1994; Biere 1995; Galloway & Fenster 2000; Joshi *et al.* 2001). However, because in most cases very few populations were studied, effects of population and habitat characteristics could not be tested at all, or not with sufficient statistical power (Kawecki & Ebert 2004).

We report results from a reciprocal replant-transplant experiment among 15 fen-grassland populations of *L. flos-cuculi* in NE Switzerland differing in population size and abiotic characteristics. In each field site, we monitored experimental transplanted plants of *L. flos-cuculi* surrounded by natural vegetation for 4 years. We ask the following specific questions: 1-

How does transplant fitness change in response to the environmental conditions at the different target sites? 2- Is fitness of the transplants influenced by characteristics of the populations of origin? 3- Is there evidence for adaptation to environmental conditions, as indicated by interactions between abiotic characteristics of transplanting sites and of sites of origin of transplants?

METHODS

Study species

Lychnis flos-cuculi L. (Caryophyllaceae) is widespread in central Europe (Hulten & Fries 1986). The species is found in sunny, nutrient-rich and moist-to-wet grasslands from the plains to the montane level. In Switzerland, it occurs naturally in moist to wet hay meadows and in degraded fens (Welten & Sutter 1982). Because of human activities, these habitats have been severely disturbed and fragmented in Switzerland during the last 150 years. As a consequence, populations of *L. flos-cuculi* have recently declined in size and number, which make them a good system to study habitat fragmentation.

Lychnis flos-cuculi is a perennial, rosette-forming herb. Seeds are able to germinate immediately after dissemination in autumn or in spring (Biere 1991). During the first growing season, a primary rosette and usually several side rosettes are formed. One or more rosettes become reproductive and grow flowering stalks of a height of 20–90 cm. The inflorescence is a dichasium consisting of up to 50 flowers, usually about 20 in the wild. Mature fruit capsules split open at the top, and up to 200 seeds are dispersed by vibrations of the stiffened stalk (Hegi 1979).

Several insects feed on *L. flos-cuculi*: vegetative plant parts are frequently attacked by snails, leaf miners and larvae of lepidopteran (Wirooks & Plassmann 1999). The moth *Coleophora silenella* (Herrich-Schäffer) lays its eggs on flower-heads of several *Silene* and *Lychnis* species

(Emmet et al. 1996). Subsequently, its larvae feed on seeds until August or September (pers. obs.).

Study sites

We chose the eight largest (> 10'000 flowering stalks, counted at the peak of flowering, in June-July 2000) and seven smallest populations (< 1'000) of a larger set of 28 populations used for various demographic and genetic studies (Galeuchet et al. 2005a, 2005b). Thus we had a wide range of population sizes to evaluate effects of fragmentation on plant performances. The 15 populations were situated in the North-East of Switzerland at altitudes of 875–1350 m (Table 1). All populations were situated in fen meadows mown yearly in September and not supplied with fertilizer. Calcareous fen meadows require nutrient-poor, base-rich, hydrological input and they therefore mostly occur in depressions with a high groundwater table or on slopes with aquifer discharges (Ellenberg 1982).

To characterize the habitat of each population, we surveyed the fen vegetation in July 2002. We recorded the identity and ground cover of all higher plant species present in a two m^2-plot situated at a random position within the *L. flos-cuculi* population. We used the species composition weighted by their ground cover to calculate mean ecological indicator values after Landolt (1977). For this study we consider the indicator values describing the ecological gradients most relevant for *L. flos-cuculi*, i.e. the light (L), moisture (F), nutrient (N) and temperature (T) indicator values. On scales from 1 (low) to 5 (high) they ranged from 2.95 to 4.27 for F, from 1.67 to 3.23 for N, from 1.88 to 3.02 for L, from 2.17 to 2.98 for T. Mean indicator values were not significantly correlated with population size ($n = 15$, F : $r = -0.11$, $p = 0.70$; N : $r = 0.00$, $p = 0.99$; L : $r = 0.24$, $p = 0.39$; T : $r = 0.18$, $p = 0.52$).

Table 1: Characteristics of the 15 study sites and populations of *Lychnis flos-cuculi*. We give municipality (Swiss canton in parentheses), name of fen, coordinates as in Swiss topographical maps, altitude, population size and mean indicator values are given. Populations were the 7 smallest (S) and 8 largest (L) of a larger set used for other demographic and genetic studies. Mean indicator values after Landolt (1977) are based on vegetation records in 2002 as explained in methods.

Site	Number of flowering stem	Altitude (m)	Coordinates		Mean indicator value for			
			E	N	Light (L)	Moisture (F)	Nutrient (N)	Temperature (T)
Bühler (AR)	40	940	751'865	250'165	2.91	3.94	2.49	0.793
Seilerzwecken (SZ)	157	1330	697'355	209'930	2.51	3.56	2.82	0.761
GäbrisWald (AR)	192	1175	753'430	249'915	2.48	3.63	2.97	0.798
Höchi (SG)	230	970	725'865	234'945	2.66	4.12	2.51	0.795
Allmeindswald (SG)	330	1080	732'220	237'525	2.99	3.68	2.66	0.812
Schlänggen (SZ)	500	900	705'586	213'240	1.88	2.95	1.67	0.77
Hasenried (SG)	800	1171	740'520	233'400	2.53	3.78	2.55	0.806
Sulzel (SZ)	2744	960	703'400	222'400	2.73	3.75	2.39	0.788
Bannholz (SZ)	11893	950	704'130	221'425	2.87	3.23	2.89	0.811
Stein (SG)	14688	950	733'655	228'725	2.68	3.58	2.6	0.789
Strandbad (SZ)	16502	900	700’950	223’100	2.8	3.75	2.5	0.811
Wideralp (SG)	18046	1350	741'465	234'620	2.52	3.94	2.53	0.797
Feusisberg (SZ)	19047	875	697'570	224'390	2.97	3.69	2.86	0.852
Feldmoos (SG)	35000	890	731'175	232'095	2.73	3.61	2.59	0.833
Munzenriet (SG)	51000	1130	744'845	229'390	2.85	3.72	2.47	0.821

We described pairwise distances between the 15 populations for geographic distance, genetic distance, and ecological (absolute indicator value) distance. We calculated geographic distances from our field recordings of site coordinates with a GPS (Global Positioning System, Garmin®, Olathe, Kansas). We obtained genetic distances between pairs of populations as F_{st} from a study of 8-18 plants per population with seven microsatellite markers (Galeuchet et al. 2005a). We evaluated absolute indicator value distance between pairs of populations as the absolute differences between population means of the F, N, L and T indicator values. These ecological 'distances' describe differences in habitat quality between sites of origin and target sites and they will be used to estimate local adaptation and phenotypic plasticity in *L. flos-cuculi*.

Plant material

In July 2000, we sampled seeds from six-eight randomly selected fruiting plants per population. Between 26 September and 19 October 2000, for each maternal family (fruit), up to 30 seeds were placed into individual pots filled with standardised horticultural soil and germinated under controlled conditions of a day/night regime of 16/8 h and a constant temperature of 25 °C. Seeds germinated within two weeks. After two weeks, we randomly selected 15 seedlings per family and transplanted them individually into small pots. The pots were kept wet in a greenhouse, where temperatures did not drop below 2°C.

We transplanted the 1350 plantlets to the field in May 2001. At each of the 15 sites we planted one offspring of each of six families per population of origin. We established six 1 m^2-plots within a 2 x 3 m area marked with metal tubes. In each 1 m^2-plot, we planted 15 plantlets at 16 planting positions in 4 rows, each with 4 plants per row separated by a 20 cm interval. One position was left unplanted as a control. We marked all plantlets with plastic rings. Per 1 m^2-plot, the 15 plantlets represented one seed family of each of the 15 populations. As eight of the 90 families had fewer than 15 plantlets, we complemented the design with a few offspring of four more families of the appropriate populations of origin.

Measurements

In August 2001, three months after transplantation, we recorded plant survival, number of rosettes and number of flowering stems per plant. In July 2002, 2003 and 2004, i.e. 14, 26 and 38 months after transplantation, we recorded plant survival, vegetative plant fitness as number of rosettes per plant and reproductive performance as number of flowering stalks. Moreover, we scored damage by herbivory on vegetative and reproductive plant parts.

Because of plant mortality, we measured 1309 plants in 2001, 1138 in 2002, 623 in 2003 and 206 in 2004, therefore our analyses lost over time. This was reflected in the higher effects observed during the two first monitoring years compared to the two last ones. The number of plants flowering in 2003 and 2004 were too low to allow testing for effects on reproductive fitness.

Data analysis

We analysed all data with hierarchical analyses of covariance (ANCOVA) on normally distributed variables (number of rosettes and number of stems) and logistic regression on binomial variable (survival from one year to the other and herbivory) with the statistical software packages JMP (Version 4.0.1, SAS Institute Inc. 2000) and GenStat 6.1.0 (Sixth Edition; ©Lawes Trust 2002).

The model contained tests for plastic plant responses to the transplanting environment, for genetic differentiation between populations of origin and maternal families, and for adaptation to conditions at the site of origin (Table 2). We tested characteristics of target sites (i.e. target-habitat quality) against residual variation among target sites, as an estimation of plastic plant response to the new environment after transplantation. The model further contained tests of effects of size and habitat quality of the population of origin against residual variation among populations of origin to estimate the effect of habitat fragmentation on plant performance. Residual variation among populations of origin was tested against variation among maternal families. We tested a home vs. away factor, because a better plant fitness for plant transplanted in their home population rather than in another population would indicate local adaptation. We also tested the effects of geographic and genetic distances between pairs of populations, and of pairwise absolute population differences in indicator values against the 'target site'-by-'site of origin' interaction. These should indicate, whether

plants perform better when transplanted closer to their site of origin, thus showing local adaptation. We then tested interactions between size of population of origin and pairwise population differences in indicator values against the 'target site'-by-'site of origin' interaction to estimate the effects of habitat fragmentation on local adaptation. In the model 37 tests (11%) were significant at the 5% level, 15 of these at the 1% level, and 11 of these at the 0.1% level, i.e. many more than expected by chance alone.

Table 2: Hierarchical ANCOVA model. This model contains effects of environmental conditions at the target site, size of population and environmental conditions of origin. Site denotes the site of the 15 populations of *Lychnis flos-cuculi*. Population size is treated as a continuous variable and Log-transformed. We calculated | Δ | values as absolutes differences between means indicator values at target site and site of origin. df = degrees of freedom, ms = mean square. For explanations of the source of variation see Methods.

Source of variation	df	ms	Variance ratio
t F	1	ms_{tF}	ms_{tF} / ms Tpop
t N	1	ms_{tN}	ms_{tN} / ms Tpop
t L	1	ms_{tL}	ms_{tL} / ms Tpop
tT	1	ms_{tT}	ms_{tT} / ms Tpop
Target pop	10	ms_{Tpop}	ms_{Tpop} / ms Pop o x T pop
Psize origin	1	ms_{PSo}	ms_{Pso} / ms Pop o
o F	1	ms_{oF}	ms_{oF} / ms Pop o
o N	1	ms_{oN}	ms_{oN} / ms Pop o
o L	1	ms_{oL}	ms_{oL} / ms Pop o
o T	1	ms_{oT}	ms_{oT} / ms Pop o
Pop of origin	9	$ms_{Pop\ o}$	$ms_{Pop\ o}$ / ms mother
Mat. plant	79	ms_{mother}	ms_{mother} / ms residual
Home	1	ms_{home}	ms_{home} / ms Pop o x T pop
Geo distance	1	$ms_{geo\ dist}$	$ms_{geo\ dist}$ / ms Pop o x T pop
F_{st}	1	ms_{Fst}	ms_{Fst} / ms Pop o x T pop
| D F |	1	$ms_{\|\ D\ F\ \|}$	$ms_{\|\ D\ F\ \|}$ / ms Pop o x T pop
| D N |	1	$ms_{\|\ D\ N\ \|}$	$ms_{\|\ D\ N\ \|}$ / ms Pop o x T pop
| D L |	1	$ms_{\|\ D\ L\ \|}$	$ms_{\|\ D\ L\ \|}$ / ms Pop o x T pop
| D T |	1	$ms_{\|\ D\ T\ \|}$	$ms_{\|\ D\ T\ \|}$ / ms Pop o x T pop
PS o x | D F |	1	$ms_{PS\ o\ x\ \|\ D\ F\ \|}$	$ms_{PS\ o\ x\ \|\ D\ F\ \|}$ / ms Pop o x T pop
PS o x | D N |	1	$ms_{PS\ o\ x\ \|\ D\ N\ \|}$	$ms_{PS\ o\ x\ \|\ D\ N\ \|}$ / ms Pop o x T pop
PS o x | D L |	1	$ms_{PS\ o\ x\ \|\ D\ L\ \|}$	$ms_{PS\ o\ x\ \|\ D\ L\ \|}$ / ms Pop o x T pop
PS o x | D T |	1	$ms_{PS\ o\ x\ \|\ D\ T\ \|}$	$ms_{PS\ o\ x\ \|\ D\ T\ \|}$ / ms Pop o x T pop
Pop o x T pop	185	$ms_{Pop\ o\ x\ T\ pop}$	$ms_{Pop\ o\ x\ T\ pop}$ / ms residual
Residual	1046	$ms_{residual}$	

RESULTS

Plastic responses to target environments

Characteristics of target sites largely affected plant performance (Table 3). At sites with higher moisture indicator value, plants grew more daughter rosettes ($F_{1,10}$=8.9, $p<0.05$) in 2003 (Fig. 1). At sites with higher light indicator value, survival was marginally significantly lower to the first year and significantly so to the second year ($F_{1,10}$=3.3, $p<0.1$, and $F_{1,10}$=5.7, $p<0.05$ respectively) and flowering plants produced fewer stems in the second year ($F_{1,10}$=20.3, $p<0.001$). At sites with higher temperature indicator value, flowering plants had a lower number of rosettes in 2001 ($F_{1,10}$=5.4, $p<0.05$) and a lower number of stems in 2002 ($F_{1,10}$=6.0, $p<0.05$). At sites with higher nutrient indicator value, plants produced more daughter rosettes in 2003 ($F_{1,10}$=5.9, $p<0.05$). Thus, as expected from a plant from wet meadows from the lower part of the montane level in Switzerland, plants of *L. flos-cuculi* performed better in more nutrient-rich and moist habitats.

Effects of characteristics of populations of origin of transplants

Size of population of origin, environmental conditions at site of origin of plants and maternal family influenced plant performance. Both in August 2001 and in July 2002, plants originating from larger field populations had grown on average more rosettes ($F_{1,9}$=5.1, $p<0.05$, and $F_{1,9}$=4.6, $p<0.1$ respectively), and in 2001, flowering plants originating from larger field population also produced more flowering stems ($F_{1,9}$=5.6, $p<0.05$), thus indicating a genetic Allee effect (Fig. 2).

Plants originating from sites with higher nutrient indicator values produced on average more rosettes in 2002 ($F_{1,9}$=7.2, $p<0.05$), while plants from sites with higher light indicator values produced marginally fewer ones ($F_{1,9}$=4.8, $p<0.1$). Plants originating from sites with higher temperature indicator values had more rosettes in 2003 ($F_{1,9}$=7.3, $p<0.05$) but survival

from 2003 to 2004 decreased ($F_{1,9}$=8.6, p<0.05). Residual variation among populations of origin and among maternal families was highly significant for several traits during the four years of survey (Appendix 1). In conclusion, both genetic and environmental background of the plants influenced their performances when transplanted to another habitat.

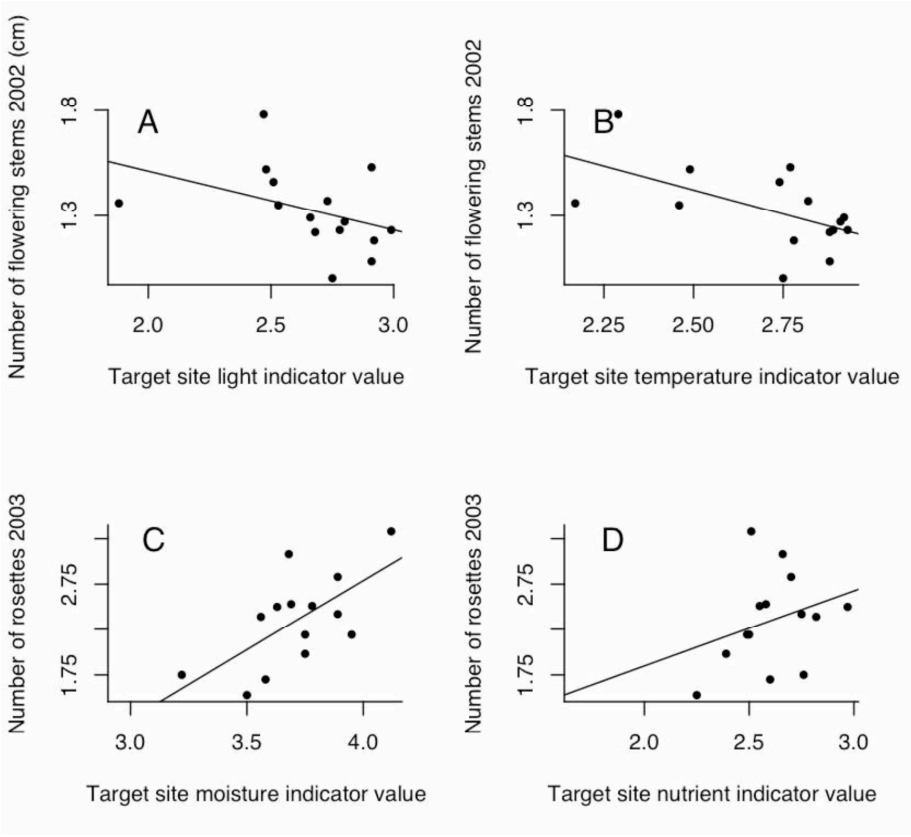

Figure 1: Relationship between transplant performance and mean indicator value (Landolt, 1977) at the 15 target sites of *Lychnis flos-cuculi*. The number of flowering stems in 2002 is negatively correlated to the target site light indicator value (p< 0.001) (A) and to the temperature indicator value (p< 0.05) (B). The number of rosettes was positively correlated to the target site moisture indicator value (p< 0.05) (C) and to the nutrient indicator value (p< 0.05) (D). Significance levels were obtained with ANCOVA (Appendix 1b and 1c). Symbols represent means over 90 plants at each of the 15 transplant target sites.

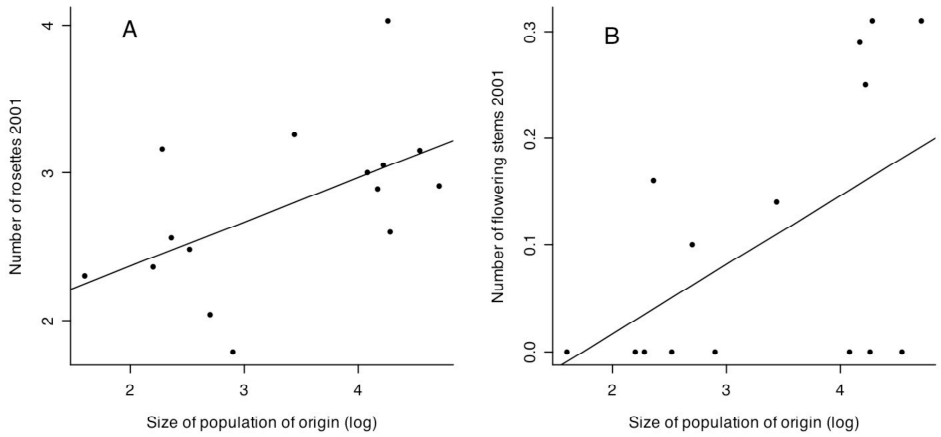

Figure 2: Positive effect of population size of origin on mean number of rosettes of *Lychnis flos-cuculi* transplants ($p < 0.05$) (A) and mean number of flowering stems ($p < 0.05$) (B) in July 2002 Significance levels were obtained with ANCOVA (Appendix 1b and 1c). We present means over 90 plants from each of the 15 population of origin.

Interactions between target sites and sites of origin

From 2001 to 2002, plants survived better in their population of origin than in other transplanted populations ($F_{1,185}=4.3$, $p<0.05$). Transplants between pairs of sites with larger geographic distance produced more flowering stems ($F_{1,185}=10.7$, $p<0.01$) and survived marginally better ($F_{1,185}=3.5$, $p<0.1$) in the first year and they produced marginally fewer rosettes in 2003 ($F_{1,185}=2.9$, $p<0.1$). Microsatellite F_{st} distances between populations did not affect plant performance.

Table 3: Estimates from the ANCOVA on the fitness-relevant traits survival, rosette number, number of flowering stems and herbivory in 2001 (I), 2002 (II) 2003 (III) and 2004 (IV) in a replant-transplant experiment with 15 populations of *Lychnis flos-cuculi*. Fitness traits are given when the statistical significance of at least one factor was p<0.1. "t" indicates characteristics of the population where the plants were transplanted, "o" indicates characteristics of the population of origin of the plants and "D" indicates the absolute difference in ecological indicator value between population of transplantation and population of origin. We used the indicator values for humidity (F), nutrient (N), light (L) and temperature (T). Positive estimates indicate a positive relationships, indicated in bold for p<0.05 and in plain for p<0.1

	Target population				Origin population				Ecological distances				
	t F	t N	t L	tT	Pop size	o N	o L	o T	Home/ Away	Geo dist	| D F |	| D N |	| D L |	| D T |
Survival I			-0.03							0.001	2.94	-2.08		
Number of rosettes I				**-0.15**	**0.3**								**3.91**	
Number of flowering stems I					**0.06**					**0.02**				
Survival II			**-0.2**						**-9.75**				**-1.83**	
Number of rosettes II					0.15	**0.62**	-0.16							**7.1**
Number of flowering stems II			**0.029**	**-0.46**										
Plants affected by herbivores II					0.02									
Survival III											-6.07			
Number of rosettes III	**1.5**	**0.8**						**0.21**		-0.09				
Survival IV								**-0.003**						**3.59**
Number of rosettes IV												2.16	-2.97	-1.38

Pairwise absolute population differences in indicator values explained significant variation for many fitness traits. Transplants between sites with higher pairwise differences in light indicator values had fewer rosettes in the first year ($F_{1,185}$=9.6, p<0.01) (Fig. 3) and a lower survival to the second year ($F_{1,185}$=6.1, p<0.05). Higher pairwise site differences in nutrient indicator values marginally significantly increased survival in the first year ($F_{1,185}$=3.0, p<0.1) and marginally decreased the number of rosettes in the fourth year ($F_{1,185}$=3.8, p<0.1). Higher pairwise site differences in temperature indicator values led to fewer rosettes in the second year ($F_{1,185}$=7.0, p<0.01), and increased plant survival from 2003 to 2004 ($F_{1,185}$=4.7, p<0.05). Higher pairwise site differences in moisture indicator

values marginally reduced survival both for 2000-2001 and 2002-2003 ($F_{1,185}$=2.8, p<0.1 and $F_{1,185}$=2.7, p<0.1). These results of reduced performance of plants transplanted to ecologically more distant sites (Appendix 1) indicate adaptation of *L. flos-cuculi* to ecological gradients.

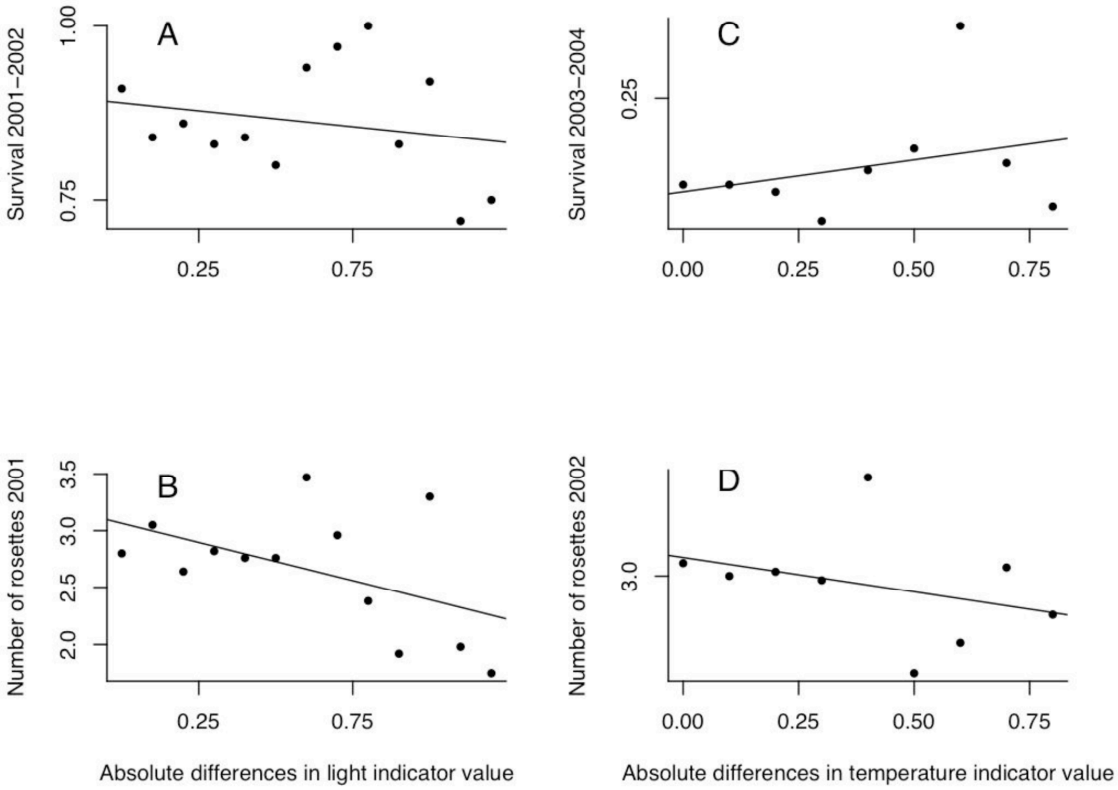

Figure 3: Effects of ecological distance between transplanting site and site of origin in a reciprocal replant-transplant experiment among 15 populations of *Lychnis flos-cuculi*. Absolute difference in ecological light indicator value, between sites of origin and target site decreased survival from 2001 to 2002 (p< 0.05)(A) and number of rosettes in 2001 (p< 0.01) (B) while absolute difference in temperature indicator value increased survival from 2003 to 2004 (p< 0.05)(C) and decreased the mean number of rosettes produced in 2002 (p< 0.01) (D). Symbols represent means per population of origin and per transplant target sites, grouped into classes of absolute differences of ecological indicator value. Significance levels were obtained with ANCOVA (Appendix 1a and 1b).

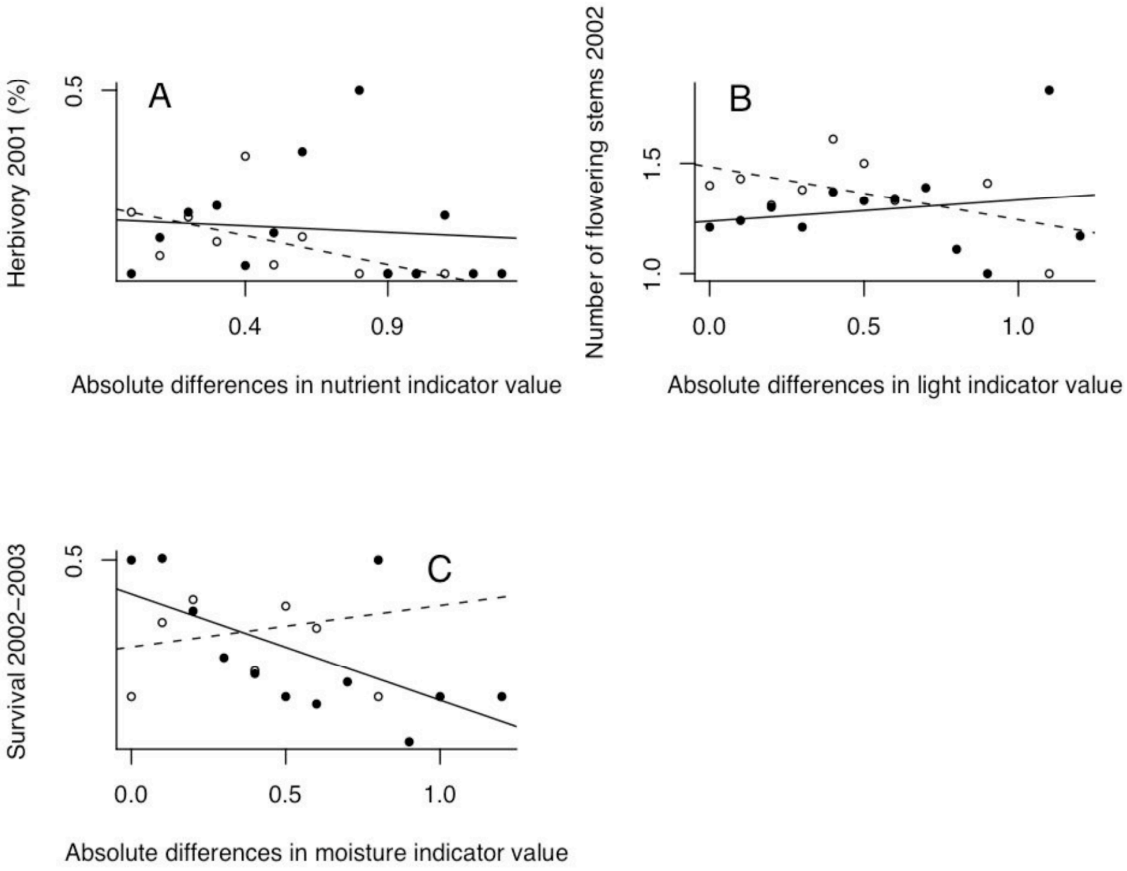

Figure 4: (A) Plants from large populations of *Lychnis flos-cuculi* were less affected by herbivores in the first year than plants originating from small populations, especially if they had been transplanted between sites with larger absolute difference in nutrient indicator values ($p < 0.05$). Symbols represent means per population of origin and per transplant target sites, grouped into classes of absolute differences of ecological indicator value. Dashed lines and open symbol represents Large populations and solid lines and filled symbols represent Small populations. Significance levels were obtained with ANCOVA. (B) Plants from large populations had a higher decrease in the number of flowering stems produced in 2002 than plants from small populations when transplanted to site with a more different light indicator value ($P<0.05$). (C) Survival from 2002 to 2003 decreased with higher pairwise absolute differences in moisture indicator value for plants from small populations ($p<0.05$) (Appendix 1d, 1c and 1a)

Plants originating from large populations were less affected by herbivores in the first year than plants from small populations, if they had been transplanted between sites with larger differences in nutrient indicator values ($F_{1,185}=5.4$, $p<0.05$)(Fig. 4) and marginally so when transplanted between sites with larger differences in moisture indicator value ($F_{1,185}=3.2$, $p<0.1$). Higher pairwise site differences in light indicator values decreased

the number of flowering stems produced in 2002 for plants from larger populations ($F_{1,185}$=5.2, $p<0.05$). Higher pairwise site differences in moisture indicator values decreased survival from 2002 to 2003 for small populations but not for large populations ($F_{1,185}$=4.5, $p<0.05$). Thus, overall, plants from large populations were better able to cope with large ecological differences between their site of origin and their transplantation site (Appendix 1).

DISCUSSION

Habitat requirements

Lychnis flos-cuculi grew best at moist sites and produced more flowers at more nutrient-rich places (Fig. 1). Generally, transplants had a higher change of performance in response to changes in moisture and light indicator, thus showing a stronger plastic response than for other indicator values. These results are consistent with an investigation in a gradient of natural soil fertility with several progeny families of *L. flos-cuculi* (Biere 1991; Biere 1996), and with clonal replicates from one population (Biere 1995). Seedling performance and components of relative growth rates showed a significant plastic response to changes in nutrient level (Biere 1995), similar to the higher number of rosettes we recorded at target sites with higher nutrient indicator value. In the earlier study, average plant biomass was lowest at the least productive site and highest in a relatively wet site with intermediate vegetation density and soil fertility (Biere 1995). Thus, based on our results and earlier work, we conclude that *L. flos-cuculi* grows best in wet habitats with moderate nutrient supply.

Genetic effects of origin of plants

We found significant variation among populations of origin and maternal seed families indicating a pronounced genetic component of plant performance. The differences between populations in vegetative growth observed after three months suggest a genetic effect of population of origin.

In two out of four years we observed differential offspring survival, growth and herbivore damage among maternal families. Our 15-population-study extends the results of a previous study of Biere (1995), who also had found part of the variation in autumn rosette biomass to be genetically based.

Higher population size positively affected growth of vegetative and reproductive plant parts (Fig. 2). We suggest that the decrease in growth we observed for plants originating from smaller populations represents a genetically based Allee effect for this fitness-relevant trait. Several common garden studies have shown positive genetic effects of increased population size on plant performance (Fischer & Matthies 1998a; Fischer *et al.* 2000; Kery 2000). In a greenhouse environment, clones of *Ranunculus reptans* from large populations produced more rosettes and flowers than plants from small populations (Fischer et al. 2000). In *Gentianella germanica* (Fischer & Matthies 1998b) and *Primula veris* (Kéry et al. 2000), survival and reproduction or growth of offspring were positively related to size and genetic variability of populations. Germination and establishment rate of *Carex davalliana* and *Succisa pratensis* were positively correlated to the size of the population of origin in a reciprocal seed transplant experiment (Hooftman et al. 2003). Seed mass and germination rate of *Ipomopsis aggregata* in the common garden (Heschel & Paige 1995) and germination rates of the perennial *Silene regia* in fragmented populations (Menges 1991) were positively related to population size. The most likely explanation for this effect is the combined action of genetic drift and inbreeding depression and the presence of the so-called Allee effect (Stephens & Sutherland 1999). To our knowledge, our study is the first to show such a genetic Allee effect in many experimental transplant sites.

Local adaptation

Evidence for adaptation to local conditions was determined based on the results of the analyses of ecological distances between sites of plant origin

and target site (Fig. 3). Local adaptation is often considered as differences in performance of natives versus immigrants or between home and away sites (Nagy & Rice 1997; Galloway & Fenster 2000). Our results indicate genetically based differences between plants from different populations in their response to different environments. Survival and vegetative growth were reduced for plants transplanted to ecologically more distant sites. In a study on *L. flos-cuculi* across four sites on a natural gradient for nutrients supply Biere (1995) found that highest reproduction and survival did not occur in the 'home' site of the clones in which the species was most abundant, but in a less productive part of the community, suggesting that adaptation to an ecological gradient is more important than adaptation to a particular site.

Damages by herbivores depended mostly on the population in which the plants had been transplanted. Here we did not find indication for adaptation of the herbivores to the plants or of the plants to the herbivores. While abiotic factors are usually rather stable, so that plant can adapt e.g. to soil conditions of light intensity, biotic factors are more dynamic. Thus there may be coadaptation, or adaptation to herbivores could be dynamic where herbivores evolve responses to any new adaptation of the plants and vice-versa, so that an "arms race" of adaptation and counteradaptation occurs (Dawkins & Krebs 1979). In our study we found no local adaptation of plant response to herbivores and the site of transplantation explained most of the variation in damages by herbivores.

Neither pairwise geographic nor selectively neutral genetic microsatellite distances between sites explained fitness variation among transplants. We conclude that habitat quality and local adaptation are more important determinants of plant performance.

Effect of habitat fragmentation on local adaptation

Patterns of survival and reproduction in transplanted sites with different ecological conditions varied significantly among plants from different populations of origin and among maternal families, indicating genetic variation in the plastic response to the environment. This suggests that the different population and maternal families have different potential to evolve in a changing environment. Some differences in plastic responses among populations of origin or maternal seed families were influenced by population size or habitat quality, suggesting that habitat fragmentation or adaptation may affect plasticity.

Plants originating from large populations reacted more strongly to different environmental conditions than plants from small populations suggesting stronger adaptation. In large populations, higher genetic diversity and higher number of individuals are favourable to the development of adaptation to local conditions, as selection needs variation to act efficiently. A 12-population-study on *Carlina vulgaris* also showed a positive effect of population size on local adaptation (Jakobsson & Dinnetz 2005). In small populations however, genetic diversity depends mostly on the effects of drift and inbreeding. As both of them tend to reduce genetic diversity, adaptation to local condition could be much more difficult due to the lack of genetic variation to act upon. Thus, while this may lead to reduced plant fitness in small populations, the performances of plants from small populations may be less affected when they are transplanted across a gradient of environmental conditions. In the longer term, however we expect plants from larger populations to perform better because the higher the genetic diversity, the higher the adaptive potential of the population.

Conclusion

We observed negative genetic effects on plant growth of small size of populations of plant origin, i.e. a genetically based Allee effect due to habitat

fragmentation. Thus, our study demonstrates that habitat fragmentation influences even the still very common *L. flos-cuculi.* Although the impact of habitat fragmentation on plant fitness was less important than that of habitat quality and of local adaptation, it cannot be neglected. Moreover, if fragmentation persists, its relevance could further increase in future decades.

Our study provides evidence that populations of the common *L. flos-cuculi* are partially adapted to their habitat and to some gradient of ecological factors. This implies that mixing seeds of different populations for ecological restoration or compensation programs should be avoided because it might break up local adaptation and seeds from population with similar ecological characteristics should be favoured.

Restoration measures are mainly concerned with small populations and it has been argued that plant material used to reinforce populations should be from populations presenting the same ecological conditions (Vergeer et al. 2004; McKay et al. 2005; Smith et al. 2005). Our study confirms this statement, and further suggest that ecological distances matter more than geographic or selectively neutral genetic distance in this respect. Moreover, the genetic diversity of the plant material used should be considered as small populations are likely to represent only little local adaptation.

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Appendix 1 Summary of analyses of A) survival, B) vegetative performance, C) reproductive performance and D) percentage of plant damage by herbivores of transplanted plants of *Lychnis flos-cuculi* ('I' denotes the measurement 3 months after transplantation, August 2001), after one year (14 months after transplantation, denoted by 'II'), after two years (26 months after transplantation, denoted by 'III') and after three year (38 months after transplantation, denoted by 'IV'). Effects comprise a set of environmental variables at target sites and sites of origin, and size of population of origin. Population denotes the 15 sites. F, N, L, T are site (t: target; o: origin) means of indicator values for moisture, nutrients, light and temperature. Due to the low number of plants surviving until 2003 and 2004, we used the mean survival per target population and per population of origin. We present mean squares (ms) or mean deviance changes (mdev), F and p values : + $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. For fitness trait explanations see methods and Table 3 (df = degrees of freedom).

A)	Survival I				Survival II				Survival III				Survival IV			
Source of variation	df	ms	F	p	df	ms	F	p	df	ms	F	p	df	ms	F	p
t F	1	0.252	1.789		1	5.342	2.552		1	4.969	1.399		1	0.562	0.144	
t N	1	0.034	0.242		1	0.371	0.177		1	1.197	0.337		1	2.345	0.599	
t L	1	0.467	3.315	+	1	11.916	5.691	*	1	9.251	2.606		1	0.398	0.102	
tT	1	0.189	1.342		1	2.205	1.053		1	1.166	0.329		1	0.237	0.060	
Target pop	10	0.141	5.499	***	10	2.094	26.521	***	9	3.551	17.179	***	10	3.913	26.031	***
Psize origin	1	0.106	1.394		1	0.092	0.283		1	0.120	0.468		1	0.022	0.321	
o F	1	0.009	0.117		1	0.062	0.190		1	0.054	0.208		1	0.109	1.611	
o N	1	0.023	0.303		1	0.120	0.369		1	0.001	0.002		1	0.130	1.913	
o L	1	0.004	0.057		1	0.098	0.301		1	0.046	0.178		1	0.077	1.135	
o T	1	0.008	0.103		1	0.023	0.070		1	0.277	1.074		1	0.582	8.562	*
Pop of origin	9	0.076	2.166	*	9	0.324	3.062	**	9	0.258	1.101		9	0.068	0.430	
Mat. plant	79	0.035	1.366	*	79	0.106	1.342	*	78	0.234	1.132		77	0.158	1.052	
Home	1	0.002	0.072		1	0.295	4.334	*	1	0.096	0.499		1	0.109	0.826	
Geo distance	1	0.092	3.551	+	1	0.052	0.769		1	0.004	0.022		1	0.114	0.863	
F_{st}	1	0.031	1.194		1	0.058	0.844		1	0.065	0.335		1	0.172	1.301	
| D F |	1	0.072	2.756	+	1	0.001	0.012		1	0.530	2.746	+	1	0.306	2.314	
| D N |	1	0.079	3.033	+	1	0.005	0.076		1	0.019	0.098		1	0.111	0.835	
| D L |	1	0.008	0.294		1	0.413	6.060	*	1	0.002	0.008		1	0.128	0.966	
| D T |	1	0.001	0.031		1	0.001	0.022		1	0.406	2.104		1	0.616	4.654	*
PS o x | D F |	1	0.003	0.104		1	0.082	1.197		1	0.876	4.538	*	1	0.338	2.551	
PS o x | D N |	1	0.009	0.361		1	0.001	0.008		1	0.019	0.097		1	0.247	1.865	
PS o x | D L |	1	0.010	0.404		1	0.001	0.009		1	0.728	3.773	+	1	0.141	1.062	
PS o x | D T |	1	0.011	0.442		1	0.010	0.143		1	0.492	2.548		1	0.000	0.001	
Pop o x T pop	185	0.026	1.014		185	0.068	0.863		171	0.193	0.934		154	0.132	0.881	
Residual	1046	0.026			1011	0.079			791	0.207			326	0.150		

B)	Number of rosettes I				Number of rosettes II				Number of rosettes III				Number of rosettes IV			
Source of variation	df	ms	F	p	df	ms	F	p	df	ms	F	p	df	ms	F	p
t F	1	3.0	0.5		1	28.2	1.4		1	37.1	8.9	*	1	4.8	1.1	
t N	1	5.2	0.9		1	8.5	0.4		1	24.5	5.9	*	1	0.4	0.1	
t L	1	3.5	0.6		1	20.7	1.0		1	1.8	0.4		1	3.0	0.7	
tT	1	30.8	5.4	*	1	1.7	0.1		1	1.9	0.4		1	0.3	0.1	
Target pop	12	5.7	1.1		11	20.4	4.2	***	10	4.2	1.5		9	4.3	1.7	
Psize origin	1	119.7	5.1	*	1	31.4	4.6	+	1	0.1	0.1		1	1.2	0.5	
o F	1	21.0	0.9		1	0.1	0.0		1	0.0	0.0		1	0.1	0.0	
o N	1	21.2	0.9		1	49.9	7.2	*	1	1.2	0.7		1	1.4	0.5	
o L	1	10.0	0.4		1	33.3	4.8	+	1	0.5	0.3		1	0.0	0.0	
o T	1	4.2	0.2		1	1.8	0.3		1	12.6	7.3	*	1	1.0	0.4	
Pop of origin	9	23.3	2.0	+	10	6.9	0.8		10	1.7	0.6		9	2.5	0.9	
Mat. plant	79	11.8	2.4	*	78	8.9	1.9	***	77	2.8	1.0		75	2.9	1.1	
Home	1	0.0	0.0		1	1.8	0.4		1	3.9	1.7		1	0.4	0.3	
Geo distance	1	3.2	0.7		1	0.0	0.0		1	6.8	2.9	+	1	1.0	0.7	
F_{st}	1	1.1	0.3		1	2.1	0.5		1	2.4	1.0		1	2.3	1.7	
| D F |	1	0.2	0.0		1	4.5	1.0		1	1.9	0.8		1	1.2	0.9	
| D N |	1	1.8	0.4		1	0.1	0.0		1	0.7	0.3		1	5.2	3.8	+
| D L |	1	41.5	9.6	**	1	2.8	0.6		1	1.0	0.4		1	4.2	3.1	+
| D T |	1	4.5	1.0		1	32.2	7.0	**	1	6.1	2.6		1	5.3	3.9	+
PS o x | D F |	1	1.3	0.3		1	3.4	0.7		1	0.0	0.0		1	0.5	0.3	
PS o x | D N |	1	9.4	2.2		1	1.2	0.3		1	4.0	1.7		1	2.4	1.7	
PS o x | D L |	1	8.2	1.9		1	0.1	0.0		1	7.5	3.2	+	1	5.2	3.8	+
PS o x | D T |	1	2.6	0.6		1	3.2	0.7		1	0.5	0.2		1	0.9	0.6	
Pop o x T pop	185	4.3	0.9		185	4.6	1.0		139	2.4	0.8		68	1.4	0.5	
Residual	1003	5.0			843	4.8			266	2.8			33	2.5		

C)	Number of flowering stems I				Number of flowering stems II			
Source of variation	df	ms	F	p	df	ms	F	p
t F	1	0.2	1.9		1	0.3	0.7	
t N	1	0.3	2.2		1	1.0	2.1	
t L	1	0.0	0.1		1	9.6	20.3	***
tT	1	0.3	2.1		1	2.8	6.0	*
Target pop	11	0.1	0.4		11	0.5	0.7	
Psize origin	1	0.7	5.6	*	1	1.6	1.4	
o F	1	0.3	2.5		1	0.7	0.6	
o N	1	0.4	2.9		1	0.2	0.2	
o L	1	0.1	0.4		1	0.4	0.3	
o T	1	0.1	0.9		1	0.2	0.2	
Pop of origin	9	0.1	1.3		10	1.2	2.0	*
Mat. plant	48	0.1	0.3		77	0.6	0.8	
Home	1	0.1	0.6		1	0.1	0.1	
Geo distance	1	1.9	10.7	**	1	0.1	0.3	
F_{st}	1	0.1	0.6		1	0.1	0.1	
| D F |	1	0.0	0.1		1	0.2	0.4	
| D N |	1	0.1	0.5		1	0.1	0.2	
| D L |	1	0.0	0.2		1	0.2	0.4	
| D T |	1	0.0	0.2		1	0.0	0.0	
PS o x | D F |	1	0.1	0.3		1	0.4	0.8	
PS o x | D N |	1	0.0	0.2		1	0.0	0.0	
PS o x | D L |	1	0.0	0.1		1	2.5	5.2	*
PS o x | D T |	1	0.0	0.0		1	1.3	2.7	
Pop o x T pop	58	0.2	0.5		155	0.5	0.7	
Residual	5	0.3			388	0.7		

D)	Herbivory I				Herbivory II				Herbivory III				Herbivory IV			
Source of variation	df	ms	F	p	df	ms	F	p	df	ms	F	p	df	ms	F	p
t F	1	0.088	0.010		1	1.107	0.176		1	0.11	0.056		1	0.088	0.726	
t N	1	0.032	0.004		1	0.267	0.042		1	3.04	1.514		1	0.102	0.840	
t L	1	3.534	0.411		1	4.035	0.640		1	1.85	0.925		1	0.058	0.478	
tT	1	0.021	0.002		1	2.150	0.341		1	0.40	0.203		1	0.098	0.810	
Target pop	10	8.590	238.038	***	10	6.304	65.747	***	9	2.01	14.78	***	8	0.122	12.03	***
Psize origin	1	0.013	0.292		1	0.231	4.545	+	1	0.03	0.416		1	0.045	1.439	
o F	1	0.053	1.158		1	0.015	0.285		1	0.00	0.046		1	0.004	0.130	
o N	1	0.001	0.025		1	0.005	0.106		1	0.00	0.007		1	0.029	0.923	
o L	1	0.006	0.125		1	0.194	3.808	+	1	0.26	2.902		1	0.053	1.689	
o T	1	0.000	0.006		1	0.489	9.603	*	1	0.25	2.810		1	0.001	0.022	
Pop of origin	9	0.046	1.842	+	9	0.051	0.695		9	0.09	0.883		9	0.031	1.145	
Mat. plant	79	0.025	0.691		78	0.073	0.764		77	0.10	0.759	+	75	0.027	2.688	*
Home	1	0.061	1.626		1	0.079	0.861		1	0.11	0.771		1	0.010	0.229	
Geo distance	1	0.057	1.524		1	0.000	0.001		1	0.01	0.045		1	0.013	0.315	
F_{st}	1	0.003	0.088		1	0.031	0.341		1	0.01	0.044		1	0.066	1.540	
| D F |	1	0.005	0.135		1	0.062	0.675		1	0.07	0.492		1	0.086	2.008	
| D N |	1	0.027	0.715		1	0.052	0.568		1	0.05	0.333		1	0.025	0.594	
| D L |	1	0.015	0.410		1	0.041	0.452		1	0.21	1.438		1	0.020	0.464	
| D T |	1	0.038	1.011		1	0.174	1.903		1	0.00	0.026		1	0.025	0.596	
PS o x | D F |	1	0.118	3.167	+	1	0.044	0.478		1	0.01	0.064		1	0.006	0.146	
PS o x | D N |	1	0.201	5.385	*	1	0.001	0.015		1	0.23	1.588		1	0.013	0.311	
PS o x | D L |	1	0.005	0.122		1	0.017	0.186		1	0.03	0.197		1	0.443	10.397	*
PS o x | D T |	1	0.007	0.178		1	0.067	0.733		1	0.07	0.509		1	0.039	0.916	
Pop o x T pop	185	0.037	1.033		184	0.092	0.955		138	0.15	1.073		67	0.043	4.218	***
Residual	1046	0.036			715	0.096			268	0.14			33	0.010		

Chapter 4

Complementarity effect among pollen donors increases offspring fitness after experimental pollination with higher pollen diversity, especially for plants from small populations

With Sophie Hoehn and Markus Fischer

KEY WORDS

Biodiversity, Caryophyllaceae, complementarity effect, effective paternity $K_{e,}$ habitat fragmentation, *Lychnis flos-cuculi*, partitioning method, pollen competition, population genetics

ABSTRACT

Multiple paternity is common in natural plant populations, enabling pollen interactions and possibly fitness effects of pollen diversity. This should be especially important in small, fragmented and therefore genetically eroded populations. We did a greenhouse pollination experiment with two levels of pollen diversity (one and four pollen donors). We used microsatellite analyses to assess paternity after four-pollen donor crosses. We used plants from eight populations to test whether large and small populations differ in effective paternity after four-donor-crosses. Moreover, we partitioned the effect of pollen paternity on offspring fitness into selection and complementarity effect. We found multiple non-random paternity after four-pollen-donor crosses. Higher experimental pollen diversity increased offspring fitness. This was due to positive complementarity effects between pollen of different donors, while selection effects were small and negative. Both non-random paternity after four-pollen-donor crosses and positive pollen diversity effects on offspring fitness were higher for plants from small than from large populations. This suggests that pollen diversity plays an important role in the context of small, fragmented populations and may be a previously overlooked mechanism for small populations to slow down genetic erosion.

INTRODUCTION

Landscape fragmentation has driven many formerly common species into small and isolated populations (Levin 1979; Saunders *et al.* 1991; van Treuren *et al.* 1991; Fischer *et al.* 2000). Small, fragmented populations are prone to genetic erosion and inbreeding (Frankham 1995). Even common species such as our study species *Lychnis flos-cuculi* show lower plant fitness and lower genetic diversity in small populations (Galeuchet *et al*. 2005a, 2005b).

In this context, pollen diversity is usually neglected. However, multiple paternity is common in natural populations (Skogsmyr & Lankinen 2002; Bernasconi 2004; Mitchell *et al.* 2005). Moreover, paternity is usually non-randomly distributed among the pollen donors (Krauss 2000). Paternity bias can occur at different steps of the pollination process, and may involve random events such as amount and timing of pollen deposition (Mitchell *et al.* 2005) or potentially strong selection via pollen competition and selective ovule abortion (Mena-Ali & Rocha 2005). Moreover, pollination by several pollen donors can increase offspring fitness (Skogsmyr & Lankinen 2002).

Effects of pollen diversity have been mainly studied under the assumption that the underlying mechanism is strong competition leading to selection for the best pollen and/or pollen donor. Pollination could allow for strong competition between pollen donors due to high numbers of pollen grains deposited compared to the number of ovules to be fertilised and this can be expected to increase offspring fitness. However, pollen diversity effects could also be due to complementarity (Bernasconi *et al.* 2003). Selection (or sampling) occurs when a larger number of pollen donors increases the probability that a "good" pollen donor is among them while complementarity occurs when the combination of several pollen donors optimises the use of resources. Bernasconi *et al*. (2003) suggested that, in pollen diversity experiments with an appropriate design, the Price-equation could be used to separate these two effects in a similar way than developed in community ecology by Loreau and Hector (2001) and refined by Fox

(2005). Our study is the first to apply these methods to analyse pollen diversity effects.

Pollen diversity can also have a strong impact on population structure and genetic diversity. Therefore, at the population level, pollen diversity can provide a powerful mechanism reducing inbreeding and favouring genetic diversity. It is also likely to alter population structure (Rigney *et al.* 1993) and could be particularly important in small genetically depauperate fragmented populations (Bernasconi 2004), where it can improve population fitness and adaptive potential. However, to our knowledge, only few studies have looked at pollen interactions in the habitat fragmentation context, and if so concentrated on cross-compatibility in self-incompatible species (Paschke *et al.* 2002).

For our model species *Lychnis flos-cuculi*, pollen interactions are highly likely as it possesses a high pollen/ovule ratio and a long style (Jurgens *et al.* 2002). Population genetic diversity and offspring fitness are positively correlated in *L. flos-cuculi* (Galeuchet *et al.* 2005b). Therefore, we expect offspring fitness to depend on pollen quality, which is prerequisite for selection or complementarity after multiple donor pollinations.

Here we report on a greenhouse pollination experiment with plants of eight small and large populations of *L. flos-cuculi* with two levels of pollen diversity (one and four pollen donors). The small populations in our study are less genetically diverse than the large populations (Galeuchet *et al.* 2005a). We assigned offspring paternity after four-pollen-donor crosses with microsatellite markers (Galeuchet *et al.* 2002) and calculated the effective paternity K_e as estimate of how skewed paternity was per maternal plant and per population. Moreover, we evaluated the effect of pollen diversity on offspring fitness. Then, we partitioned the fitness effects of pollen diversity with the recently published method from community ecology to separate selection effects from complementarity effects (Fox 2005). More specifically, we addressed the following questions: 1- Does non-random paternity occur in our populations and does it lead to increased offspring fitness? 2- Is increased offspring fitness achieved by selection

and/or by complementarity? 3- Are skewness of paternity and fitness effects of pollen diversity and its components different between small and large populations?

METHODS

Study species

The ragged robin *Lychnis flos-cuculi* (= *Silene flos-cuculi* (L.) Clairv., *Coronaria flos-cuculi* (L.) Braun) (Caryophyllaceae) is common throughout most of Europe, except for the Artic region. It grows in sunny, wet-to-moist habitats such as fens and wet meadows and can be found from the plain to the montane level.

This rosette forming, perennial herb reproduces sexually and clonally. It produces several flowering stems bearing up to 50 flowers in dichasial inflorescences. Flowers are protandrous and the plants are mainly outcrossed (Biere 1996), but selfing also occurs. Its main pollinators are Diptera and Hymenoptera (Vejsnæs & Høvsgaad 1990). The five stigmas expose receptive hair on most of their length, providing a large surface to deposit and spread pollen and the pollen/ovule ratio is high, 192 $\pm$23 (Jurgens *et al.* 2002). This is in line with the mainly outbreeding mating system, which indicates that it is a good model species to study pollen competition. In our study area, plants flower in June-July and the fruits are mature in August. Fruit capsules contain up to 200 seeds, mostly dispersed close to the maternal plant. Seeds germinate in autumn and in spring (Biere 1991).

Although *L. flos-cuculi* is still common, its populations have been declining in number and size in the last decades due to changes in agriculture. The species is used in restoration measures in Switzerland such as extended field margins in the agricultural landscape and meadow restorations (Lehmann *et al.* 2000). Thus it is a good model to study habitat fragmentation and the related conservation issues.

Study sites

For our study we selected plants of four small (<4000) and four large (>14000) populations (Table 1) situated in North-Eastern Switzerland, which belong to a larger demographic study (Hoehn *et al*, in prep). Molecular genetic data are available for these 8 populations from a study of 14 to 28 plants per population with seven microsatellite primer pairs (Galeuchet *et al.* 2005a). Among the eight populations, expected microsatellite heterozygosity (H_e) varied from 0.825 to 0.859, allelic richness (AR) from 4.56 to 5.2 and inbreeding coefficient (F_{is}) from 0.316 to 0.525.

Table 1: Characteristics of the eight study sites and populations of *Lychnis flos-cuculi* from which the experimental plants originated. We give municipality (Swiss canton in parentheses: SG, St-Gallen, SZ, Schwyz and AR, Appenzell Ausserrhoden), coordinates as in Swiss topographical maps, altitude, population size, isolation and genetic characteristics (AR: microsatellite allelic richness, H_e: expected microsatellite heterozygosity, F_{is}: inbreeding coefficient). Populations were four of the smallest (S) and four of the largest (L) of a larger set used for other genetic (Galeuchet et al., 2005a) and demographic studies (Galeuchet et al., 2005b). Population size denotes the number of flowering individuals in 2000.

Site	Coordinates E	Coordinates N	Altitude (m)	Pop. Size	Number of flowering individuals	AR	H_e	F_{is}
Gäbriswald(AR)	753'430	249'915	1175	S	192	4.83	0.817	0.525
Hausmösl1(AR)	740'920	242'250	940	S	920	4.7	0.853	0.452
Sulzel(SZ)	703'400	222'400	975	S	2700	4.56	0.834	0.472
Chellen(SG)	730'380	237'310	1080	S	3800	5.2	0.857	0.427
Landscheidi(AR)	734'640	240'580	940	L	14100	4.8	0.825	0.316
Obermoos(SZ)	697'570	224'390	870	L	19000	4.97	0.848	0.451
Feldmoos(SG)	731'175	232'095	890	L	35000	4.94	0.859	0.521
Munzenriet(SG)	744'845	229'390	1030	L	51000	4.82	0.854	0.403

Plant material

We used 40 four-year-old plants, five from each of the eight populations. Plants were grown in the greenhouse from seeds sampled in the field in

summer 2000 and sown in September 2000. We kept the plants above 0°C in winter, where they received natural light, and water according to needs. We chose the plants depending on flowering synchronicity within population samples, all plants coming from different maternal seed families. To prevent self-fertilisation, we emasculated the two-to-four-day-old flowers used as recipients once they opened. Moreover, to prevent insect pollination, we covered them with fine nylon mesh. We used only fresh pollen for all crosses.

Crosses

In spring 2004, we performed 40 four-donor crosses and 102 single donor crosses using plants both as pollen donors and as recipients. We did all crosses among plants originating from the same population.

For the 102 one-pollen-donor crosses, we brushed a stamen over each stigma of the recipient flower to saturate them with pollen. We estimated the number of pollen deposited by spreading pollen on three control flowers. For these flowers we cut one of five stigmas out, squashed it gently on a slide with a drop of water and counted the number of pollen grains under a binocular. We multiplied it by five to obtain the number of pollen grains per flower. On average we counted 2600 ($\pm$ 1041 S.E.) pollen grains on each flower. We could not perform 58 single donor crosses due to insufficient flowering synchronicity among plants from the same population.

For the four-pollen-donor crosses, we spread the sticky pollen donor by donor. We counted the pollen grains under a binocular and spread them evenly over the sticky hairs of the five receptive stigmas of the recipient plant with a pin. We used about 100 pollen grains per stigma and per pollen donor, i.e. about 2000 for 200 ovules per flower; we saturated all stigmas with pollen. The time between the deposition of the first pollen grains from the first pollen donor and the last pollen grains of the fourth pollen donor was 30 to 60 minutes. We included the order of the pollen deposition from each donor, different for each cross, as covariate in the analysis and found it unrelated to percentage of offspring sired ($N= 226$, $F= 0.081$; $P=0.78$).

Measurements

In spring 2004, we collected the seeds, and counted and weighed them per fruit. We sowed up to 30 seeds per fruit in September 2004 in multi-pot trays with 3 x 3 cm pots (15 seeds per pot) containing standardised soil substrate (BF4, Tref de Baat, Coevorden, The Netherlands) and scored germination after four weeks. In October 2004, among the six-week-old plantlets we randomly selected up to five offspring for each single donor cross and up to 20 offspring for each multiple donor cross. We transplanted them individually into 10 cm diameter pots filled with the same substrate. In spring 2005, when most plants flowered, we measured fitness for all plants, 331 plants from single donor crosses and 416 plants from multiple donor crosses. To estimate their fitness, we counted the number of rosettes, flowering stems and flowers. In spring 2005, we harvested the plants above ground, dried them for 24h at 80°C and weighed them to the nearest 10 μg on an electro balance.

Paternity analysis

We screened the seedlings of the multiple donor crosses with microsatellites. We used up to 5 primer pairs in order to assign a pollen donor to each of up to 20 seedlings per fruit. We used offspring grown in the greenhouse and additionally sown offspring to increase the number of offspring available for the paternity screening. 334 of 436 screened plants could be unambiguously assigned to a pollen donor. We did not use the remaining 102 plants non-assigned plants in further analyses.

Data analysis

Realised paternity

To assess whether pollen donor performance was consistent across maternal plants, we tested whether the mean siring-success rank differed between pollen donors. As an estimation of paternity skewness, we calculated effective paternity K_e (Starr 1984) as $K_e = 1/\Sigma(pi)^2$, where pi is the

percentage of offspring sired by pollen donor. To see whether paternity was non-random, we tested whether K_e values were different from four. To see if the K_e values depended on the populations and genetic variation within these populations, we used ANCOVA, where we tested the effect of expected heterozygosity H_e against remaining variation among populations and the latter against the residual variation.

Offspring fitness

From the fitness data, first we calculated the coefficient of variation (CV) per fruit as the standard deviation divided by the mean per fruit with up to five offspring per one-pollen-donor cross and up to 20 offspring per four-pollen-donor cross for each fitness measurement, and then the average CV of all fitness traits. We expected the variation in fitness traits to be higher if more different pollen donors had sired offspring. With an ANCOVA we tested the effect of population characteristics and number of pollen donors on both fitness traits and coefficient of variation. We transformed data prior to analyses to fit the assumption of normality. We analysed binomial variables such as survival and flowering with logistic regressions and we obtained F-values by dividing mean deviance due to a factor by their appropriate error mean deviance, analogous to the calculation of F-values in ordinary analysis of variance (Payne *et al.* 1993). We tested the population characteristics against remaining variation among populations and population, treatment (one- and four-pollen-donor cross) and the interactions population size x number of pollen donors against the interaction population x number of pollen donors, the interaction population x number of pollen donors against the interaction maternal plant x number of pollen donors and finally maternal plant and the interaction maternal plant x number of pollen donors against the residual variation. We also used ANCOVA to test whether the pollen donor siring most seeds produced the offspring with the highest fitness.

Separating complementarity and selection effects

As suggested by Bernasconi *et al*. (2003), we partitioned the effect of pollen diversity on offspring fitness into selection effect and complementarity effect. This requires information on the proportion of seeds of a maternal plant sired by each pollen donor in a multiple pollen donor cross, and on plant performance after single donor crosses for each pollen donor used in the multiple donor cross on the same maternal plant. When a pollen donor was missing in the one pollen donor cross (no seed produced or no cross performed), the offspring of this pollen donor could not be analysed with this method, as we could not estimate the expected fitness trait, and we had to omit all offspring of this pollen donor. Those pollen donors represented in the one-pollen-donor cross, that did not sire offspring in a multiple donor cross, were assigned a fitness trait of 0 for this multiple donor cross. Because several one-pollen-donor crosses could not be performed, we finally used 134 plants across 34 maternal plants (1-13 offspring per maternal plant).

Net effect, complementarity effect, and selection effect

We analysed the diversity effects with the statistical package R according to Fox (2005). We calculated the net effect (NE) of multiple donor crosses as the difference between the observed fitness trait among offspring of the multiple donor crosses and its expected value under the null hypothesis that there is no pollen diversity effect. This expected value is the weighted (with initial relative proportion of pollen of the pollen donor in the multiple donor cross, here 0.25) average of the fitness trait among offspring of the one pollen donor cross for the pollen donors of the multiple pollen donor cross.

This net effect value is then partitioned into two effects, the complementarity (CE) and selection effect (SE) (Loreau & Hector 2001), where the selection (sampling) effect must be corrected for a trait-dependent effect (TDCE) before it can be considered as analogous to natural selection (Fox, 2005). We calculated selection effect and

complementarity effect per maternal plant. SE is measured as the covariance between the fitness trait of the offspring of one pollen donor in the one-pollen-donor cross and the change in relative fitness trait between multiple-pollen-donor crosses and single-pollen-donor crosses. It is positive if pollen donors with higher-than-average fitness trait sire most seeds, at the expense of the other pollen donors. A positive CE occurs when trait fitness of offspring from the multiple donor cross are on average higher than expected on the basis of the weighted average of the fitness trait of the offspring from the one pollen donor cross for the components pollen donors, independently of the pollen donor traits and not at the expense of the other pollen donors. The third additive partition TDCE is not relevant here and we calculate it only as correction for SE.

Statistical tests

We tested net, complementarity and selection effects with a t-test to see if they were different from 0 and with an ANCOVA to find out if they depended on population, population size, and number of pollen donors actually producing offspring. We tested population size against remaining variation among populations, and population, number of pollen donors producing offspring, and the interaction population size x number of pollen donor producing offspring against the residual variation.

RESULTS

Paternity

In our multiple pollen donor crosses, some of the most successful pollen donors sired more than twice the expected number of seeds while the least successful ones did not sire any seeds (Table 1). Among 33 maternal plants effective paternity K_e was between 1 and 3.846 and differed significantly from 4 (mean: 2.17, $t_{32}=15.07$, $p<0.001$) where 1 indicates highly skewed paternity (only 1 pollen donor out of 4) and 4 random paternity (each pollen donor siring one fourth of the offspring). K_e increased significantly with H_e of the population of origin ($F_{1,5}=7.9$, $P<0.01$), indicating that the

less genetically diverse a population the more skewed paternity was (Fig. 1).

The rank of siring success differed between pollen donors ($F_{37,65}$=5.8, P<0.001). However, maternal plant identity did not affect pollen donor ranking, indicating that selection for the best pollen donor cannot alone explain the observed paternity ($F_{26,65}$=0.23, NS).

Table 2: Number of offspring sired in four-pollen-donor crosses (expected number of sired offspring in bracket) for the 40 pollen donors from eight small and large populations of *Lychnis flos-cuculi* in a greenhouse pollen diversity experiment. Crosses are pooled per population and per pollen donor across five maternal plants per population. The expected number of sired offspring varied because the number of offspring identified per cross varied.

Population	Pollen donors				
Obermoos(SZ)	**1**	**2**	**3**	**4**	**5**
36 offspring	11 (9)	6 (8)	0 (4)	19 (8)	0 (7)
Gäbriswald(AR)	**6**	**7**	**8**	**9**	**10**
50 offspring	5 (9.5)	11 (8)	7 (11)	19 (11.5)	8 (10)
Sulzel(SZ)	**11**	**12**	**13**	**14**	**15**
21 offspring	8 (4.25)	2 (5.75)	5 (3.25)	4 (7.25)	2 (3.5)
Feldmoos(SG)	**16**	**17**	**18**	**19**	**20**
49 offspring	8 (8)	4 (10.75)	22 (11)	5 (10)	10 (9.25)
Munzenriet(SG)	**21**	**22**	**23**	**24**	**25**
45 offspring	4 (7.75)	5 (10)	11 (9)	11 (11.25)	14 (7)
Landscheidi(AR)	**26**	**27**	**28**	**29**	**30**
58 offspring	10 (10.25)	16 (13.5)	3 (11)	26 (11.25)	3 (12)
Chellen(SG)	**31**	**32**	**33**	**34**	**35**
40 offspring	4 (7.5)	9 (7.75)	8 (10)	4 (6)	15 (8.75)
Hausmösli(AR)	**36**	**37**	**38**	**39**	**40**
49 offspring	16 (9.75)	18 (8.5)	4 (8.5)	5 (12.25)	6 (4)

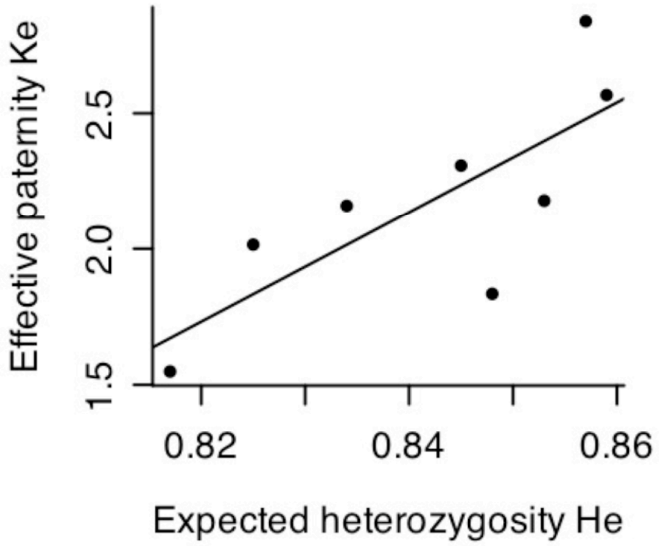

Figure 1: Effective paternity K_e in relation to heterozygosity in a greenhouse pollen diversity experiment (one and four pollen donors) with plants of eight large and small populations of *Lychnis flos-cuculi* ($p<0.05$). $K_e = 1$ denotes highly skewed paternity and $K_e = 4$ random paternity.

Offspring fitness in multiple-sired fruits

In the greenhouse, offspring of the most successful pollen donor did not have higher fitness than those sired by less successful pollen donors (Appendix 1a), and even had marginally significantly fewer and shorter leaves at six weeks, and fewer flowering stems. Thus, the ability to sire a high percentage of seeds was independent of the quality of the resulting offspring. Pollen donor, maternal plant, and population identity had a significant effect on offspring fitness assessed as number of flowering stems, number of vegetative rosettes, and biomass.

We then tested for an advantage of offspring fitness from fruits produced by multiple donor crosses in comparison to one-pollen-donor crosses. Seed were significantly heavier in the multiple-sire fruits ($F_{1,44}=24.6$, $P<0.001$), leaves at six weeks were more numerous ($F_{1,673}=4.3$, $P<0.05$) (Fig. 2) and germination rate was marginally significantly higher. Only the number of flowering stems was marginally significantly lower among the offspring of the four-pollen-donor crosses. In interaction with the identity of the maternal plant, the number of pollen donors also had an effect on seed mass ($F_{18,44}=2.6$, $p<0.01$), number of leaves ($F_{19,673}=3.1$, $p<0.001$) and longest leaf size at six weeks

($F_{19,671}$=4.4, p<0.001), number of vegetative rosettes ($F_{19,669}$=2.4, p<0.001) and number of flowering stems ($F_{19,671}$=1.9, p<0.05) (Appendix 1b). In conclusion, fitness of offspring sired by multiple pollen donors was generally higher than of offspring sired by only one pollen donor.

The mean coefficient of variation (CV) of the components of vegetative and reproductive fitness marginally significantly increased with the applied number of pollen donors ($F_{1,34}$=4.1, p=0.09)(Appendix 1c). This increase differed between populations ($F_{6,34}$=3.5, p<0.05).

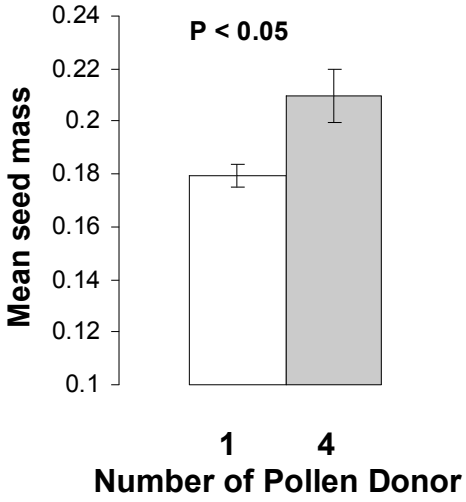

Figure 2: Mean seed mass depending on the type of cross in a greenhouse pollen diversity (one and four pollen donors) experiment with plants from eight small and large populations of *Lychnis flos-cuculi*. The white bar represents 102 one-pollen-donor crosses and the grey bar represents 40 four-pollen-donor crosses.

Separation of the effect of selection and complementarity

We then studied the effects of pollen diversity on offspring fitness with the partitioning method. The net diversity effect was marginally significantly different from 0 for the number of leaves and longest leaf length at 6 weeks and the number of vegetative rosettes (t_{23}=1.8; t_{23}=2.0 and t_{24}=1.9 respectively, p<0.1) (Table 3a). For all measures the positive net effect indicated positive effects of higher pollen diversity.

We partitioned the net effect into complementarity effect and selection effect. For all measures the complementarity effect was positive. The complementarity effect was significantly different from 0 for the number of leaves (df=18, t=4.6, p<0.001) and longest leaf length (t_{18}=4.6, p<0.001) at six weeks, number of vegetative rosettes (t_{19}=3.1, p<0.01) and biomass (t_{19}=3.5, p<0.01).

Table 3 Statistical analyses performed on the net effect NE, complementarity effect CE, and selection effect SE in the pollen diversity (one and four pollen donors) experiment with plants from eight small and large populations of *Lychnis flos-cuculi* for six fitness traits. The t-test (A) shows whether there is an effect of pollen diversity and whether it is due to selection or to complementarity. The ANCOVA (B) indicates which factors influenced these same effects. The selection effect SE was never significant in the ANCOVA and was removed. Due to missing data, the complementarity effects on the number of flowering stems and number of flowers were analysed for six populations only. Level of significance: **+** < 0.1; * < 0.05; ** < 0.01; *** < 0.001

A)	Nb of leaves at 6 weeks			Leaf length at 6 weeks			Number of flowering stems		
t-test	NE	CE	SE	NE	CE	SE	NE	CE	SE
df	23	18	18	23	18	18	24	13	13
t	1.8 +	4.6 ***	-2.9 **	2 +	4.6 ***	-3.6 **	1.1	1.6	-0.04

A)	Number of flowers			Number of vegetative rosettes			Biomass		
t-test	NE	CE	SE	NE	CE	SE	NE	CE	SE
df	24	13	13	24	19	19	24	19	19
t	1.1	1.8 +	-0.09	1.9 +	3.1 **	-0.9	1.6	3.5 **	-2.3 *

B)	Nb of leaves at 6 weeks				Leaf size at 6 weeks				Number of flowering stems				Number of flowers				Number of vegetative rosettes				Biomass			
	NE		CE		NE		CE		NE		CE		NE		CE		NE		CE		NE		CE	
ANOVA	df	ms	df	ms	df	ms	df	ms	df	ms	df	ms	df	ms	df	ms	df	ms	df	ms	df	ms	df	ms
Pop. Size	1	5.2	1	0.1	1	121	1	23	1	5.9 ***	1	4.8	1	7	1	780.5	1	13.3	1	123	1	1.9	1	4.5
Population	6	26.3	6	20.1	6	130	6	208	6	0.1	4	1.1	6	276	4	626.9	6	48.0	6	54.2	6	3.7	6	3.9
Nb of donors	1	151 *	1	128 *	1	646 +	1	669 *	1	13.8 +	1	11.7 *	1	3001	1	1184.9	1	174.3	1	0.6	1	28.9 +	1	39.3 *
Nb of donors * Pop. Size	1	34.9	1	19.0	1	186	1	205	1	5.9	1	2.6	1	492	1	1393.7	1	59.3	1	28.2	1	7.6	1	7.0
Residual	14	32.8	9	22.5	14	163	9	126	15	3.9	6	1.0	15	1387	6	417.7	15	90.8	10	138	15	8.0	10	6.0

The selection effect was generally 10 times smaller than the complementarity effect. The selection effect was significantly different from 0 for the number of leaves (t_{18}= -2.9, $p<0.01$) and longest leaf length (t_{18}=-3.6, $p<0.01$) at six weeks and biomass (t_{19}=-2.3, $p<0.05$). The selection effect was negative for all measures. To summarise, the net diversity effect was positive for all fitness traits and was composed of a positive complementarity effect and a weak negative selection effect.

The higher the realised number of sires, the larger was the net diversity effect for the number of leaves at 6 weeks ($F_{1,14}$ =4.6, $p<0.05$) (Fig.3). The net effect increased marginally significantly with the realised number of sires for longest leaf length at six weeks ($F_{1,14}$ =3.9, $p<0.1$), number of flowering stems ($F_{1,15}$ =3.5, $p<0.1$) and biomass ($F_{1,15}$ =3.6, $p<0.1$) (Table 3b). Moreover, complementarity effects significantly increased for the same four vegetative traits number of leaves at six weeks ($F_{1,14}$ =5.6, $p<0.05$), longest leaf size at six weeks ($F_{1,14}$ =5.3, $p<0.05$), number of flowering stems ($F_{1,15}$ =11.7, $p<0.05$) and biomass ($F_{1,15}$ =6.5, $p<0.05$), while selection effects tended to decrease (Fig.3). Opposite, but non-significant trends appeared for the reproductive traits. For the number of flowering stems and the number of flowers, the complementarity effects tended to decrease with the realised number of sires, while selection effects tended to increase. In summary, net and complementarity effect increased or tended to increase with higher number of pollen donors siring seeds

Plants from small populations had a significantly higher net effect than plants from large ones for the number of flowering stems ($F_{1,15}$ =72.5, $p<0.001$)(Fig. 4). The same trend was not significant for the other measures (Table 3b). The complementarity effect tended to be larger for plants from small populations for all measures but the number of vegetative rosettes, while no trend could be found for the selection effect. Thus, net and complementarity effects increased or tended to increase with decreasing population size.

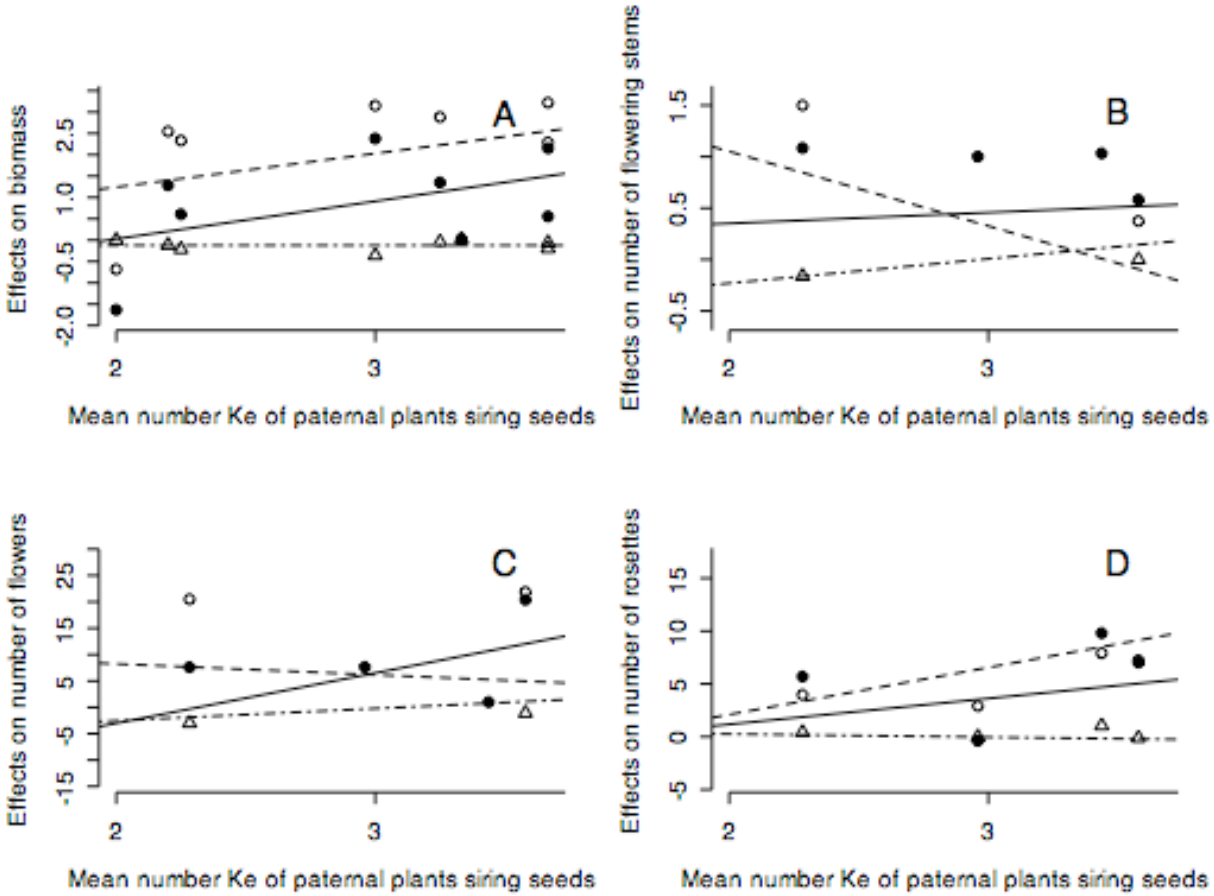

Figure 3: Effect of pollen diversity (one and four pollen donors) on plant fitness in a greenhouse pollen diversity experiment with plants from eight small and large populations of *Lychnis flos-cuculi*. The net effect (solid line, black circles) of pollen diversity is stronger for offspring from fruits effectively sired by more paternal plants. We give the fitness traits biomass (A), number of flowering stems (B), number of flowers (C) and number of rosettes (D). The net effect can be partitioned into complementarity effect (dashed line, white circles) and selection effect (dashed-dotted line, white triangles) (Fox 2005). Here, these two effects show opposite slopes. The slope of the complementarity effects (dashed line, white circles) of biomass (A) and of number of flowering stems (B) are significant while the slope of the net effects (solid line, black circles) of biomass (A) and of number of flowering stems (B) are marginally significant.

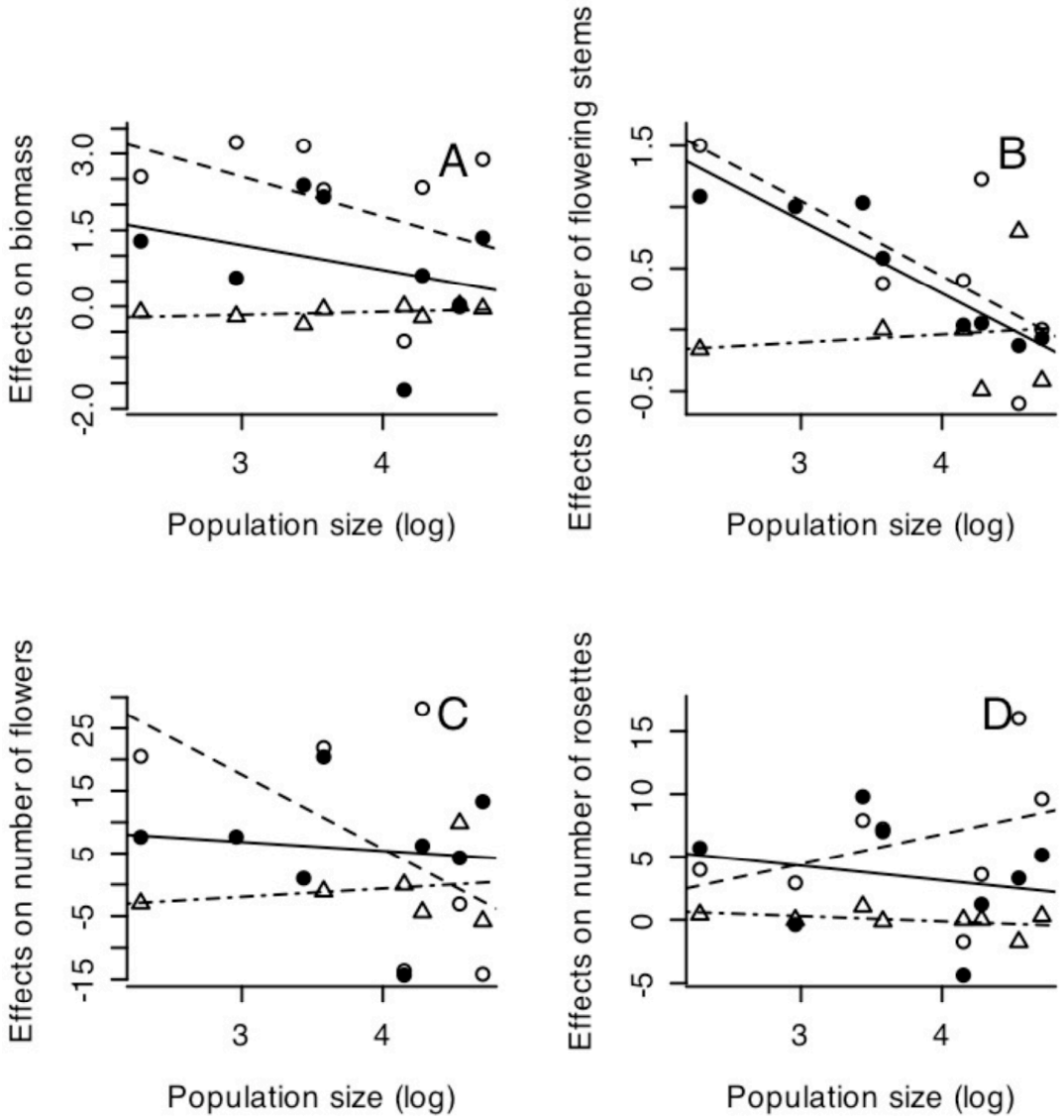

Figure 4: Effect of pollen diversity (one and four pollen donors) on plant fitness in a greenhouse pollen diversity experiment with plants from eight small and large populations of *Lychnis flos-cuculi*. The net effect (solid line, black circles) is stronger for plants from small than large populations for several fitness traits: biomass (A), number of flowering stems (B), number of flowers (C) and number of rosettes (D). The net effect can be partitioned into complementarity effect (dashed line, white circles) and selection effect (dotted dashed line, white triangles) (Fox 2005). Here, these two effects show opposite slopes. Only the slope of the net effect of number of flowering stem (solid line in B) is significant ($p<0.001$).

DISCUSSION

Non-random paternity

In our populations, multiple donor pollination led to highly skewed multiple paternity in the offspring. In contrast to other studies (Snow & Spira 1996; Marshall 1998; Mitchell & Marshall 1998), we found that the ranking of successful pollen donors was only partly constant. First, this could be due to the partly different pollen combinations in each cross due to permutation of four out of five possible pollen donors, which could create differences in male-male interactions (Cruzan 1990; Marshall 1998). Second, females might have influenced the male-male interactions and even exerted a choice depending on compatibility, genetic distance, or gene quality (Paschke *et al.* 2002). In any case, it is important to note that selection for the best pollen cannot explain the observed paternity pattern.

Although non-random multiple paternity has been found in other experiments (Taylor *et al.* 1999; Krauss 2000), this is the first time that it has been studied across several populations of different sizes. Paternity was more skewed in plants from less heterozygous small populations than in plants from more heterozygous large populations. To our knowledge, this is the first time that such a relationship has been shown. It implies that plants from large populations were better able to profit from the diversity of arriving pollen whereas plants from small populations seem to be less able to discriminate against a strong sire. Alternatively, the overall quality of paternal plants and deposited pollen might be lower and more variable in small populations and may favour a stronger choice for the best pollen or pollen donor.

We found a trend towards a higher variance of fitness traits in the fruits sired by several pollen donors rather than by one pollen donor. Higher variance after multiple-pollen-donor crosses has also been found in earlier studies (Kress 1981; Winsor *et al.* 2000; Banuelos & Obeso 2003). Even if offspring fitness in the greenhouse would not be increased after pollination with more diverse pollen, having more diverse progeny might

still be advantageous to cope with a heterogeneous environment (Yasui 1998), so that diverse paternity as such could be promoted as a bet-hedging strategy.

Pollen diversity increases offspring fitness

Multiple paternity increased offspring fitness, especially seed mass. Some earlier studies also found a benefit of multiple pollen donors (Walsh & Charlesworth 1992; Marshall *et al.* 2000).

Offspring of the most successful pollen donor did not have higher fitness than offspring of less successful pollen donors. An alternative explanation is that the maternal plants rather provision the ovules fertilised first, so that the fastest pollen sire the fittest offspring, whatever their genetic quality (Delph *et al.* 1998). In any case, in our experiment selection did not appear to be important in explaining advantages of multiple paternity for offspring fitness (also see next section).

Complementarity versus selection effect

We separated selection and complementarity in the effects of pollen diversity on offspring fitness (Loreau & Hector 2001; Fox 2005). This is the first time that this technique of analyses has been used outside community ecology.

With this method we confirmed that the selection effect played only a marginal role in comparison to the complementarity effect. Moreover, both effects were even antagonistic. Clearly, the positive overall net effect was ruled by positive complementarity, while negative selection played a minor role. This contradicts the usual assumption that a combination of pollen competition and a positive relationship between siring ability and offspring fitness is the major mechanism after multiple pollination.

The level of diversity used in experiments can shape the observed biological response. In most previous studies only two pollen donors were used (Bernasconi 2004 but see Krauss 2000), while here pollen of four

donors was interacting. Thus our experiment was more likely to detect effects of multiple-paternity. Moreover, complementarity increased with the effective paternity K_e.

Fitness effects of pollen diversity in the context of habitat fragmentation

Plants in small populations are more likely to have accumulated deleterious mutations because of genetic drift, have lower genetic diversity and heterozygosity and/or are expected to be more related to each other (Galeuchet *et al.* 2005a). Paschke (2002) reported that an increase in the number of pollen donors increased the reproductive success of plants from one small and one large population of the self-incompatible species *Cochlearia bavarica* due to increased cross-compatibility. Here, small populations benefited more from complementarity than large populations. Moreover, several fitness traits showed a higher net and complementarity effect for the smaller population.

There are several potential mechanisms. In small populations, plants have lower fitness compared to plants from large population because of higher inbreeding and lower genetic diversity. Therefore, plants from small populations have a potentially higher fitness gain than plants from large populations. Higher pollen diversity could allow these plants to compensate for detrimental effects of small population size if they are able to select genetically different pollen. They could also favour diversity of sires as such as a bet-hedging strategy, which could reduce the chance that all their offspring would be homozygous for the same deleterious allele.

Conclusion

Our study adds a new level of biodiversity to the traditionally studied species and genetic biodiversity so far addressed in studies of functional consequences of biodiversity. The next step will be to identify the exact

mechanisms underlying the observed positive pollen diversity effects on paternity and on offspring fitness.

This is also the first study to compare pollen diversity effects in plants from large and small populations, and we have shown here that pollen diversity plays an important so far overlooked role in the context of small, fragmented populations. Pollen diversity effects may well be an important mechanism for small populations to slow down genetic erosion.

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Appendix 1a: ANCOVA of offspring fitness in relation to percentage of offspring sired per pollen donor in a greenhouse pollen diversity (one and four pollen donors) experiment with plants of eight small and large populations of *Lychnis flos-cuculi*. The percentage of offspring a pollen donor sired in a four-donor cross was only marginally and negatively correlated with offspring fitness. Level of significance: **+** < 0.1; * < 0.05; ** < 0.01; *** < 0.001

		Number of leaves at 6 weeks		Longest leaf length at 6 weeks		Number of stems		Number of flowers		Number of vegetative rosettes		Biomass	
Source of variation	DF	ms		ms		ms		ms		ms		ms	
Population size	1	1.58		205.26		1.87		56.30		0.03		3.004	
Population	6	9.23		116.72		9.86	*	1851.79	+	60.35	*	8.116	**
Pollen donor	27	6.36	***	66.59	***	3.40	***	678.68	**	11.58	+	1.329	*
Percentage sired	1	16.89	+	199.56	+	10.27	+	1250.82		17.72		3.126	
Maternal plant	21	5.14	***	59.13	***	3.05	***	792.35	**	20.55	***	2.052	***
Residual	170	2.46		21.27		1.19		341.82		7.49		0.796	

Appendix 1b: ANCOVA of fitness measures in a greenhouse pollen diversity (one and four pollen donors) experiment with plants of eight small and large populations of *Lychnis flos-cuculi*. Fitness increased with the higher experimental pollen diversity. Level of significance: + < 0.1; * < 0.05; ** < 0.01; *** < 0.001

Source of Variation		Number of seeds		Mean seed mass		Germi-nation		Number of leaves		Longest leaf length at 6 weeks	
	DF	ms		ms		ms		DF	ms	ms	
Population Size	1	611.6		0.019		0.33		1	32.9	713.2	+
Population	6	4228.7		0.01	**	0.18	*	6	32	+ 185.9	
Maternal plant	31	3137.5	**	0.003	***	0.06	+	30	15.7	*** 178.7	***
Number of donor	1	642.2		0.016	***	0.12	+	1	10.6	* 1.03	
Number of donor * Population Size	1	9748	+	0.003		0		1	0.2	33.54	
Population * Number of donor	6	2149	+	0.002		0.04		6	5.4	120.3	
Maternal plant * Number of donor	18	897.5		0.002	**	0.03		19	7.6	*** 96.5	***
Residual	45	1159.9		0.001		0.04		673	2.4	22.06	

Source of Variation		Number of stems		Number of flowers		Number of vegetative rosettes		Biomass	
	DF	ms		ms		ms		ms	
Population Size	1	0.22		1.4		1.07		12.64	
Population	6	3.19	*	23.6	***	140.78	**	9.25	**
Maternal plant	30	1.14	***	4.3	**	29.52	***	2.61	***
Number of donor	1	0.92	+	0.7		17.07		0.5	
Number of donor * Population Size	1	0.13		2.7		2.51		0.02	
Population * Number of donor	6	0.63		4.3		14.97		2	
Maternal plant * Number of donor	19	0.58	*	3		17.62	***	1.36	+
Residual	673	0.31		2.5		7.36		0.94	

Appendix 1c: ANCOVA of the average coefficient of variation per fruit assessed with five offspring per fruit of one-pollen-donor crosses and 20 offspring per fruit of four-pollen-donor crosses for six fitness traits in a greenhouse pollen diversity (one and four pollen donors) experiment with plants of eight populations of *Lychnis flos-cuculi*. Level of significance: +< 0.1; * < 0.05

Source of variation	DF	ms	P
Population size	1	0.0002	
Allelic Richness	1	0.0512	
F_{is}	1	0.1247	
Population	4	0.0616	
Maternal plant	29	0.0340	
Number of donor	1	0.1901	**+**
Number of donor * Population size	1	0.0014	
Number of donor * Population.	6	0.0466	*****
Number of donor * Maternal plant	16	0.0135	
Residual	34	0.0344	

Chapter 5

Offspring fitness after inter-population crosses of the common *Lychnis flos-cuculi* L. in relation to the geographic, genetic and ecological distances between populations

With Sophie Hoehn and Markus Fischer

Key Words

Caryophyllaceae; co-adapted gene complex; habitat fragmentation; hybrid vigour; Landolt indicator values; local adaptation; *Silene Flos-cuculi*

Abstract

We evaluated the consequences of between-population crosses for plants of 13 populations of the common but declining *Lychnis flos-cuculi.* Previous information on molecular diversity and ecological conditions allowed us to study the roles of genetic diversity, and of genetic and ecological, along with geographical, distances between populations for offspring fitness. We crossed 7 plants of each of the 13 populations with a plant from: 1/ the same population, 2/ a small, 3/ a large, 4/ an ecologically similar and 5/ an ecologically dissimilar population to obtain the F1 generation. We grew plants of this F1 generation until flowering both in the greenhouse and in the field populations of origin. Moreover, we used the greenhouse plants to perform F2 and backcross crosses. Offspring of crosses between populations had higher vegetative and reproductive cumulative fitness in the F1 both in the greenhouse and in the field, indicating heterosis rather than breaking-up of local adaptation. F1 performance also increased with larger geographic and ecological distances between parental populations. Although the heterosis effect was still positive in the F2 and backcrosses, it was not significant any more. Within-population crosses tended to be more beneficial for populations with lower allelic richness for most fitness traits and generations. Nevertheless, overall our study suggests that heterosis after between population crosses outweighs potential negative consequences.

INTRODUCTION

Habitat fragmentation reduces population size and gene flow between populations. Small populations have reduced genetic diversity because of inbreeding and genetic drift (Barrett & Kohn 1991; Ellstrand & Elam 1993). Linked with the loss of genetic diversity and increased inbreeding, habitat fragmentation is also often associated with a reduction in plant fitness in small populations (Young *et al.* 1996; Fischer & Matthies 1998; Kolb 2005), the so-called Allee effect (Stephens *et al.* 1999). To counteract these effects, genetic rescue by artificial gene flow may be a valuable tool (Ingvarsson 2001; Tallmon *et al.* 2004; Hedrick 2005). Recreating gene flow disrupted by landscape fragmentation introduces new alleles to threatened populations, thus reducing inbreeding and increasing fitness of crosses between different populations relative to within-population crosses (Edmands 1999; Vergeer *et al.* 2004; Willi & Fischer 2005).

However, crosses between populations can also lead to outbreeding depression. Crosses between genetically distant populations can break-up co-adapted gene complexes (Lynch 1991). Alleles can behave differently depending on the genomic context they belong to and beneficial epistatic genetic effects can be disrupted. Moreover, plant populations often show strong local adaptation in relation to differences in local ecological conditions (Schmitt & Gamble 1990; Linhart & Grant 1996; Joshi *et al.* 2001; Hufford & Mazer 2003; Kawecki & Ebert 2004). Therefore, the effects of mixing of gene pools can also be detrimental. Indeed, break-up of local adaptation has been found in several studies on inter-population crosses (Waser 1993b; Fischer & Matthies 1997).

Differences between populations play an important role in predicting consequences of crosses between populations. These differences can be estimated in different ways. First, larger geographical distances between populations may be proxies for larger genetic and environmental differences. Second, selectively neutral molecular genetic distances directly indicate how closely related populations are. Third, quantitative

genetic distances indicate how genetically similar populations are in ecologically relevant traits. Fourth, distances in ecological conditions may be proxies for differences due to local adaptation.

To evaluate the consequences of local adaptation and fragmentation for fitness after inter-population crosses, we used the common but declining *Lychnis flos-cuculi.* We performed inter-population crosses among 13 populations differing in size and ecological conditions and grew the resulting offspring in the greenhouse and in the parental natural populations. Moreover, we produced F2 and backcrosses to study longer-term consequences of inter-population crosses. We wanted to answer the following questions: 1- Is offspring fitness larger after crosses between populations than within populations and how does this change across generations? 2- Are benefits of between-population crosses higher for plants from smaller populations? 3- Do consequences of between-population crosses change with geographical, genetic, and ecological distances between populations?

METHODS

Study species

The ragged robin *Lychnis flos-cuculi* (= *Silene flos-cuculi* (L.) Clairv., *Coronaria flos-cuculi* (L.) Braun) (Caryophyllaceae) is common throughout most of Europe. It grows in sunny, wet-to-moist habitats such as fens and wet meadows and can be found from the plain to the montane level.

This rosette forming, perennial herb reproduces both sexually and clonally and produces several flowering stems, bearing up to 50 flowers in dichasial inflorescences. The flowers are protandrous, but while plants are mainly outcrossed (Biere 1996), selfing still occurs. The main pollinators are Diptera and Hymenoptera (*Bumbus*) (Vejsnæs & Høvsgaad 1990). In our study area, plants flower mainly in June-July and fruits mature in August. Fruit capsules contain up to 200 seeds, which are dispersed principally around the maternal plant by vibration of the stiffened stalk. The seeds are able to germinate in autumn and in spring (Biere 1991).

Although still common, populations of *L. flos-cuculi* have been declining in the last decades due to changes in agriculture and urbanisation, and therefore it is a good model for studying the effects of habitat fragmentation. From an applied point of view, mixing gene pools of *L. flos-cuculi* is interesting because it is often used in restoration measures in Switzerland such as sown wild flower strips in the agricultural landscape (Lehmann *et al.* 2000).

Table 1: Characteristics of the 13 study sites and populations of *Lychnis flos-cuculi*. We give municipality (Swiss canton in parentheses), coordinates as in Swiss topographical maps, altitude, population size and mean ecological indicator values. Population size denotes the number of flowering stems in 2000. Populations were the 6 smallest (<800) and 7 largest (>2500) of a larger set used for other demographic and genetic studies. Mean ecological indicator values after Landolt (1977) are based on vegetation records in 2002 as explained in methods. We obtained allelic richness from a study of 8-18 plants per population with 7 microsatellite markers (Galeuchet, 2005b). We used Schlänggen (SZ) population for crosses in the greenhouse but we did not transplant plants to this site.

Site	Pop. Size	Alti-tude (m)	Coordinates		Allelic Richness	Mean indicator value for			
			E	N		Light (L)	Moisture (F)	Nutrient (N)	Acidity (R)
Bühler (AR)	40	940	751'865	250'165	4.77	2.91	3.94	2.49	2.91
Seilerzwecken (SZ)	157	1330	697'355	209'930	4.46	2.51	3.56	2.82	2.77
Gäbris Wald (AR)	192	1175	753'430	249'915	4.83	2.48	3.63	2.97	2.88
Allmeindswald (SG)	330	1080	732'220	237'525	4.98	2.99	3.68	2.66	2.12
Schlänggen (SZ)	500	900	705'586	213'240	4.63	1.88	2.95	1.67	1.86
Hasenried (SG)	800	1171	740'520	233'400	4.69	2.53	3.78	2.55	3.05
Sulzel (SZ)	2744	960	703'400	222'400	4.56	2.73	3.75	2.39	2.11
Bannholz (SZ)	11893	950	704'130	221'425	4.91	2.87	3.23	2.89	2.59
Stein (SG)	14688	950	733'655	228'725	4.54	2.68	3.58	2.6	1.87
Strandbad (SZ)	16502	900	700’950	223’100	4.78	2.8	3.75	2.5	2.04
Feusisberg (SZ)	19047	875	697'570	224'390	4.97	2.97	3.69	2.86	2.6
Feldmoos (SG)	35000	890	731'175	232'095	4.94	2.73	3.61	2.59	2.37
Munzenriet (SG)	51000	1130	744'845	229'390	4.82	2.85	3.72	2.47	1.65

Plant origin

Our 13 populations of plant origin belong to a larger set of populations studied in a demographic study in North-Eastern Switzerland (Hoehn *et*

al. in prep). In 2001, population size of the six small (<800) and seven large (>2500) populations ranged from 40 to 51 000 flowering stems (Table 1) and allelic richness of these populations varied from 4.46 to 4.98. To characterize the habitat of each population, we surveyed the fen vegetation in July 2002. We recorded the identity and ground cover of all higher plant species present in a two m^2-plot situated at a random position within the *L. flos-cuculi* population. From the species composition weighted by ground cover we calculated mean ecological indicator values after Landolt (1977) for each habitat. For this study we consider the indicator values describing the ecological gradients most relevant for *L. flos-cuculi*, i.e. the light (L), moisture (F), nutrient (N) and soil acidity (R) indicator values. On scales from 1 (low) to 5 (high) they ranged from 2.95 to 4.27 for F, from 1.67 to 3.23 for N, from 1.88 to 3.02 for L, and from 1.65 to 3.05 for R. Mean indicator values were not correlated with population size (n = 15, F: r = -0.11, p = 0.7 ; N: r = 0.00, p = 0.99; L: r = 0.24, p = 0.39; R: r = -0.28, p = 0.29). The variation in the ecological conditions between its populations makes *L. flos-cuculi* a good study species for local adaptation.

We assessed pairwise geographic, genetic, and ecological distances between the 13 populations. We calculated geographic distances from our field recordings of site coordinates with a GPS (Global Positioning System, Garmin, Olathe, Kansas, U.S.A.). We obtained molecular genetic distances between pairs of populations as F_{ST} and allelic richness for each population from a study of 8-18 plants per population with seven microsatellite markers (Galeuchet *et al.* 2005a). We calculated mean quantitative trait distances Q_{st} between pairs of populations (Merila & Crnokrak 2001) with the data from Galeuchet (2005b). We assessed ecological distance between pairs of populations as the absolute differences between population means of the F, N, L, and R indicator values to describe differences in habitat quality between parental populations.

Crosses

For our crosses, we started with two-year-old plants grown from seeds sampled in the field in July 2000 and sown in September 2000 in the greenhouse (Perret 2003; Galeuchet *et al.* 2005b). We kept all plants above 0°C in winter and provided natural light, and water according to needs.

First generation (F1)

In spring 2003, we performed within- and between-population crosses with offspring of 13 natural populations. We produced four types of between-population crosses: with large, small, ecologically similar and ecologically dissimilar (according to ecological indicator values for light and moisture) populations. We used 107 plants to perform 410 crosses, resulting in 281 fruits (Fig. 1A).

In August 2003, we counted the number of seeds and sowed up to 30 seeds per fruit in multi-pot trays with 15 seeds per 3 x 3 cm pot containing standard substrate (BF4, Tref de Baat, The Netherlands). We scored germination after four weeks. In September 2003, we randomly selected three plantlets per fruit and transplanted them individually to 10-cm-diameter pots filled with the same substrate. We kept them in a greenhouse (921 plants). We also transplanted four additional plantlets per fruit to 12 of our natural populations (991 plants), two to each parental population. In summer 2004, we monitored F1 plants in the greenhouse and in the field. We measured plant survival, the size and numbers of vegetative and reproductive parts, and we additionally assessed plant damage by pathogens and herbivores in the field.

Second generation (F2) and backcrosses to the maternal population (Ba)

In spring 2004, we performed 323 F1 x F1 crosses (287 fruits) to obtain F2 plants from within and between-population crosses (Fig. 1A). For each population of origin, we crossed only F1 plants from the same of the five F1 cross types to obtain F2. The F1 within-population crosses were

crossed among themselves for each population of origin as control. We also performed 202 backcrosses of within-population F1 and of between-population F1 with the original maternal plants resulting in 172 fruits (Fig. 1B).

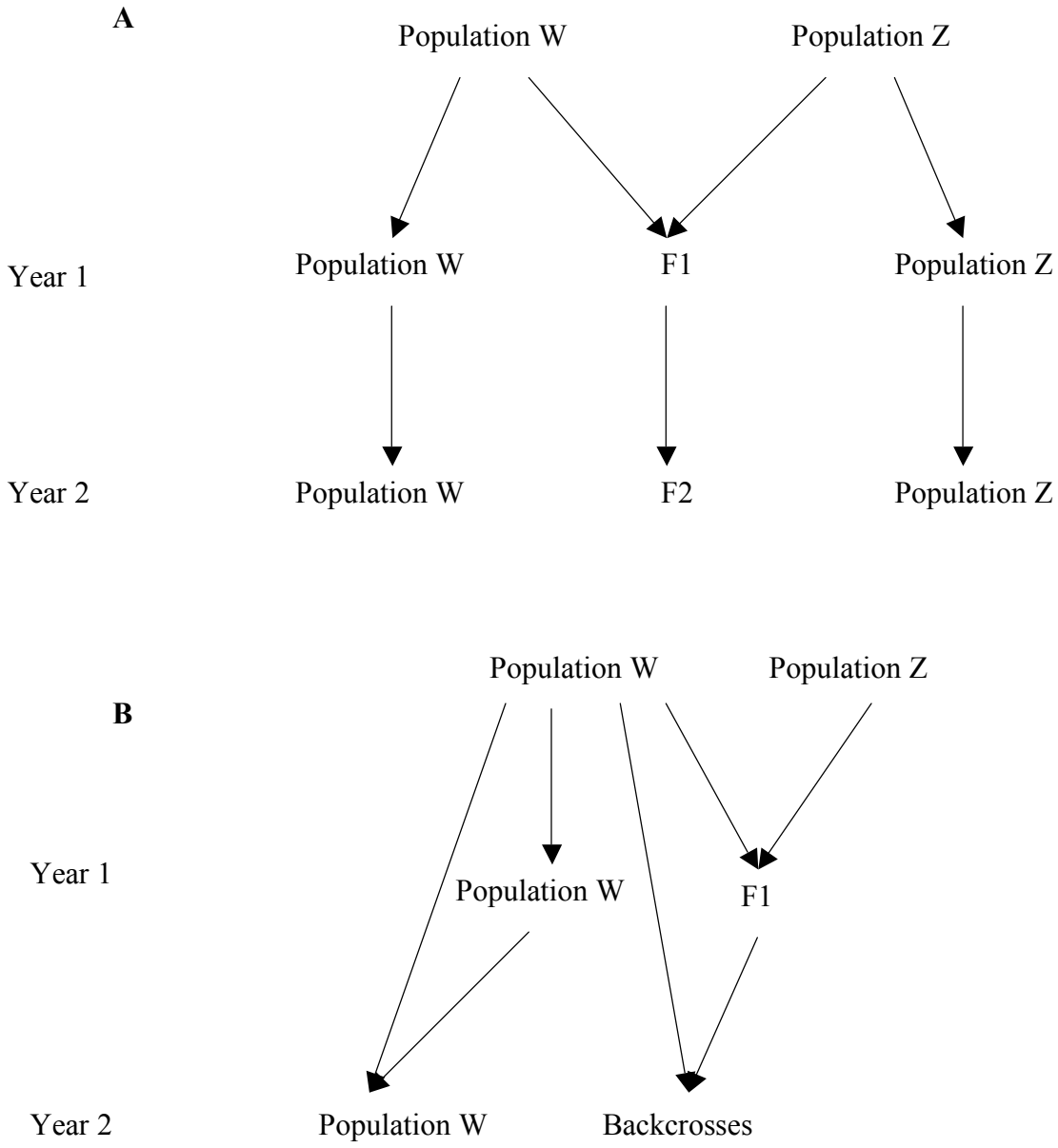


Figure 1 Schemes of the crosses performed in the greenhouse with plants from pairs of 13 natural populations of *Lychnis flos-cuculi*.

In September 2004, we counted the resulting seeds and sowed up to 30 seeds per fruit in multi-pots trays with 15 seeds per 3 x 3 cm pots

containing standard substrate. Again, we scored germination after four weeks. In October 2004, we randomly selected two plantlets per fruit and transplanted them in the same way in the greenhouse than the F1 (1294 plants). We additionally grew F2 and backcrosses in competition with *Anthoxanthum odoratum* (20 commercial seeds added during transplantation to 10-centimeter-diameter pots; 918 *L. flos-cuculi* plants). We used competition to test whether effects of between-populations crosses change with plant stress. F2 reproductive fitness decreased significantly with competition ($F_{1,334}$=233, $p<0.05$) in accordance with previous results (Galeuchet *et al.* 2005b). In summer 2005, we measured the number of rosettes and of flowers, and survival.

Data analysis

We used logistic regression to analyse binomial data (Survival) with the statistical package R. We divided mean deviances due to a factor by their appropriate error mean deviances, analogous to the calculation of F-values in ordinary analysis of variance (Payne *et al.* 1993). We analysed vegetative (number of seeds x germination rate x number of rosettes) and reproductive (number of seeds x germination rate x number of flowers) cumulative fitness, both square root transformed to meet the assumption of normality, with hierarchical analyses of covariance (ANCOVA) with the statistical software package JMP (Version 4.0.1, SAS Institute Inc. 2000).

For the F1 in the greenhouse and in the field, we tested for effects of allelic richness of maternal population, population size and ecological indicator values (humidity, acidity, nutrient, light) to examine the role of habitat fragmentation characteristics on plant performances. We also tested effects of paternal population characteristics. We tested the effects of within- versus between-population crosses and of the four different between-population crosses to assess the consequences of different types of crosses. We tested effects of neutral and quantitative genetic distances between populations (pairwise F_{st} and Q_{st} values) and effects of

geographic distances between populations and also the absolute difference between indicator values of maternal and paternal populations. Then we tested effects of the interaction between allelic richness of the maternal population with the treatments. We also tested for the effects of corresponding interactions of the allelic richness of paternal populations (see Table 2 for ANCOVA model). To analyse the performance of the F1 transplanted to the field, we added transplant site as source of variation and tested it against the residual (see Table 3 for ANCOVA model). To analyse the F2 and backcrosses, we used a similar but slightly reduced model (see Table 4 and 5 for ANCOVA model).

RESULTS

Characteristics of parental populations

In the greenhouse F1 offspring from maternal populations with higher allelic richness had higher vegetative (number of seeds x germination rate x number of vegetative rosettes) and reproductive (number of seeds x germination rate x number of flowers) fitness, indicating genetic Allee effects ($F_{1,7}$=8.7, $p<0.05$ and $F_{1,7}$=10.6, $p<0.05$ respectively; i.e. Fig. 2).

For the F1 transplanted to the field, vegetative plant performance decreased significantly with increasing maternal population size ($F_{1,6}$=11.3, $p<0.05$). Plant performance also depended on several characteristics of the paternal population. Reproductive fitness increased with acidity indicator value of the paternal population ($F_{1,6}$=6.1, $p<0.05$). Survival increased with humidity ($F_{1,6}$=6.4, $p<0.05$) and light ($F_{1,6}$=8.5, $p<0.05$) indicator value, and decreased with nutrient indicator value ($F_{1,6}$=7.9, $p<0.05$) of the paternal population. Thus, characteristics of maternal and paternal populations explained variation in plant performance when transplanted to the field.

Residual variation among maternal and paternal families and populations was highly significant for most cumulative fitness measures and generations, confirming a strong genetic component of plant

performance. At the same time, for all three fitness traits of the F1 in the field, the site of transplantation was a significant source of variation.

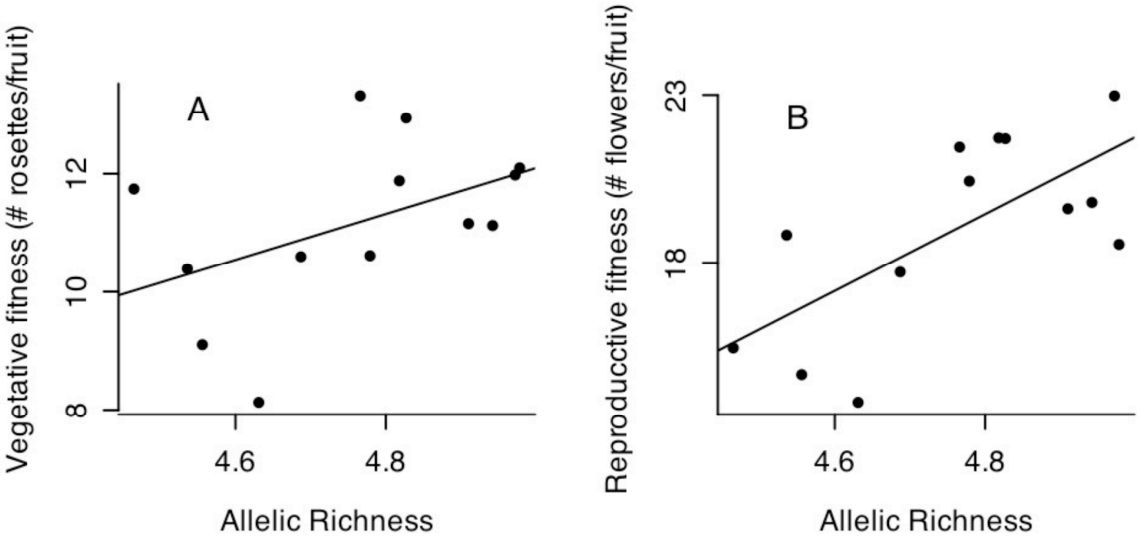


Figure 2: Allee effect in offspring of crosses within and between populations of *Lychnis flos-cuculi.* Positive effect of maternal population allelic richness on vegetative ($p<0.05$) (A) and reproductive ($p<0.05$) (B) cumulative fitness in F1 plants in the greenhouse, all crosses included. Levels of significance were obtained with hierarchical ANCOVA (Table 2). Vegetative and reproductive fitness denote the total number of offspring rosettes or flower resulting per original fruit after one generation.

Within versus between population crosses

F1 in the greenhouse

F1 plants from between-population crosses had significantly higher vegetative ($F_{1,22}=8.2$, $p<0.01$) and reproductive ($F_{1,23}=7.4$, $p<0.05$) fitness in the greenhouse than plants from within-population crosses (Fig. 3), showing a clear heterosis effect with all four between-population cross types.

▶▶

Table 2: Analyses of covariance for vegetative and cumulative fitness of F1 plants in the greenhouse after crosses within and between populations with plants from 13 natural populations of *Lychnis flos-cuculi*. Factors and covariates comprise a set of parental population characteristics, cross types, and genetic, geographic, and ecological distances between these populations. Population denotes the 13 parental populations. We square root transformed the data to meet the assumption for normality. We present mean squares (ms), F and p values: + $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Cross types are S:Small, L:Large, Si:Similar and Di:Different.

F1 fitness in the greenhouse	Vegetative				Reproductive			
Sources of variation	DF	ms	F	P	DF	ms	F	P
Maternal Pop. A.R.	1	390.7	8.67	*	1	2817.1	10.63	*
Maternal Pop size	1	250.6	5.56	+	1	61.1	0.23	
Maternal Pop. Humidity (F)	1	99.1	2.20		1	552.7	2.08	
Maternal Pop. Acidity (R)	1	0.1	0.00		1	41.5	0.16	
Maternal Pop. Nutrient (N)	1	227.0	5.04	+	1	593.4	2.24	
Maternal Pop. Light (L)	1	23.1	0.51		1	145.8	0.55	
Maternal Population	7	45.0	0.45		7	265.1	0.79	
Maternal plants	86	99.8	14.89	***	86	336.9	50.30	***
Paternal Pop. A.R.	1	40.3	0.60		1	180.2	0.80	
Paternal Pop Size	1	98.8	1.47		1	590.9	2.62	
Paternal Pop. Humidity (F)	1	0.7	0.01		1	49.7	0.22	
Paternal Pop. Acidity (R)	1	0.2	0.00		1	147.0	0.65	
Paternal Pop. Nutrient (N)	1	9.3	0.14		1	11.1	0.05	
Paternal Pop. Light (L)	1	77.6	1.16		1	553.3	2.45	
Paternal Population	7	67.2	1.33		7	225.8	1.30	
Paternal plant	93	50.3	7.51	***	93	173.9	25.97	***
Cross (W. vs B.)	1	310.2	8.21	**	1	1041.3	7.40	*
Cross Type (S, L, Si, Di)	3	121.6	3.22	*	3	283.4	2.01	
Geo. Dist	1	22.9	0.61		1	155.8	1.11	
Fst	1	6.1	0.16		1	19.1	0.14	
Mean Qst	1	39.8	1.05		1	315.0	2.24	
Humidity (F) Dist.	1	21.7	0.57		1	1.4	0.01	
Acidity (R) Dist.	1	1.5	0.04		1	18.3	0.13	
Nutrient (N) Dist.	1	149.2	3.95	+	1	246.6	1.75	
Light (L) Dist.	1	184.3	4.88	*	1	898.6	6.39	*
Cross x Maternal Pop. A.R.	1	54.1	1.43		1	131.1	0.93	
Cross Type x Maternal Pop. A.R.	3	26.2	0.69		3	73.8	0.52	
Geo. Dist x Maternal Pop. A.R.	1	4.1	0.11		1	6.7	0.05	
Fst x Maternal Pop. A.R.	1	2.5	0.07		1	0.1	0.00	
Cross Type x Paternal Pop. A.R.	3	34.8	0.92		3	31.3	0.22	
Geo. Dist x Paternal Pop. A.R.	1	1.8	0.05		1	5.2	0.04	
Fst x Paternal Pop. A.R.	1	23.7	0.63		1	0.4	0.00	
Maternal Pop x Paternal Pop	22	37.8	5.64	***	23	140.7	21.01	***
Residual	631	6.7			617	6.7		

F1 in the field

The F1 in the field from between-population crosses had higher reproductive fitness ($F_{1,7}$=8.5, p<0.001) than plants from within-population crosses and marginally significantly higher vegetative fitness ($F_{1,17}$=4.1, p<0.1). Thus, F1 plants in the field also showed heterosis.

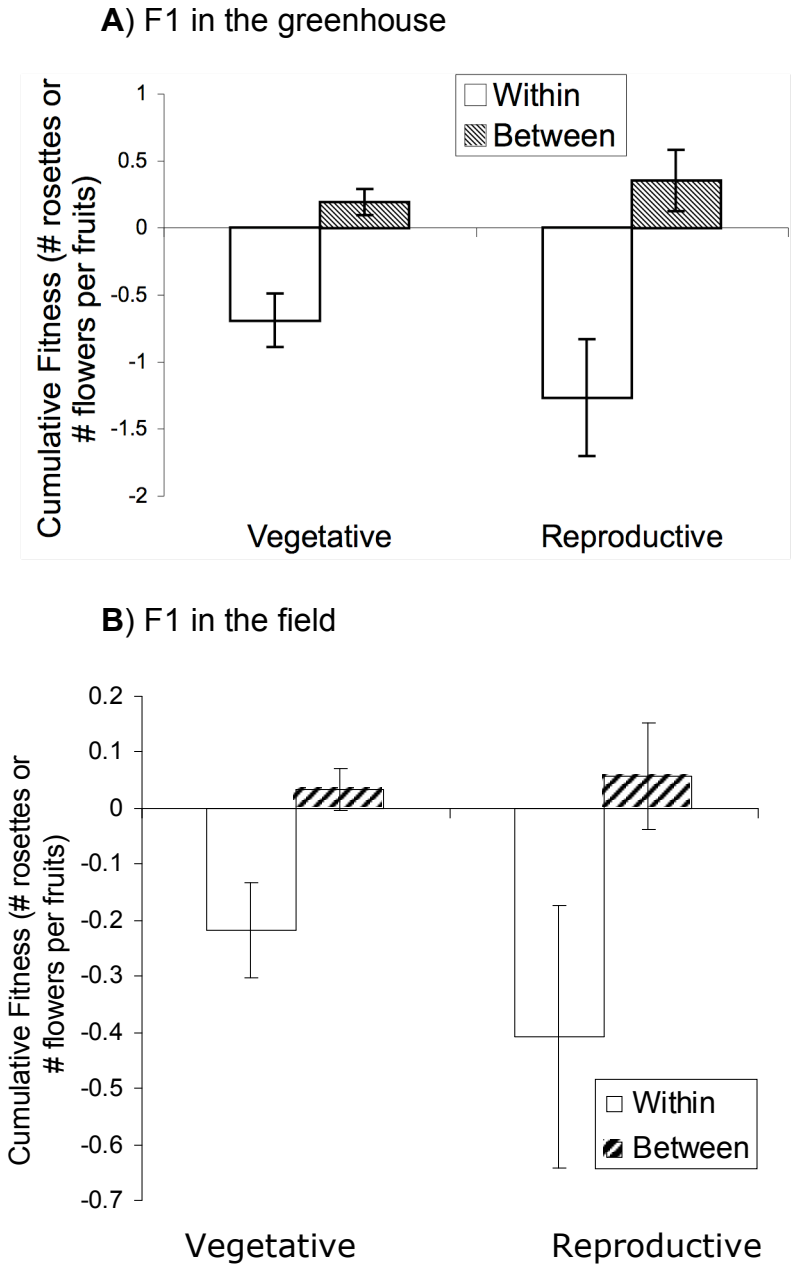


Figure 3: Means of residual fitness of the 13 natural populations of *Lychnis flos-cuculi* for offspring of within and between population crosses. F1 plants from between population crosses had significantly higher vegetative (p<0.01) and reproductive (p<0.05) fitness in the greenhouse (A) and higher vegetative (p<0.1) and reproductive (p<0.001) fitness in the field (B). Levels of significance were obtained with hierarchical ANCOVA (Table 2 and 3). Bars indicate one S.E.

F2 in the greenhouse

F2 fitness after between-populations crosses was not significantly higher than after within-population crosses. However, the interaction competition x cross indicated that F2 vegetative fitness with competition was marginally significantly higher in plants from between than within-population crosses while this was not the case without competition ($F_{1,441}$=2.85, p<0.1; i.e. Fig. 4). The effects of between- versus within-population crosses depended on the population for all traits.

Plants from populations with lower allelic richness had a marginally significantly higher vegetative fitness when crosses were performed within populations (cross x maternal allelic richness: $F_{1,7}$=4.64, p<0.1) rather than between populations.

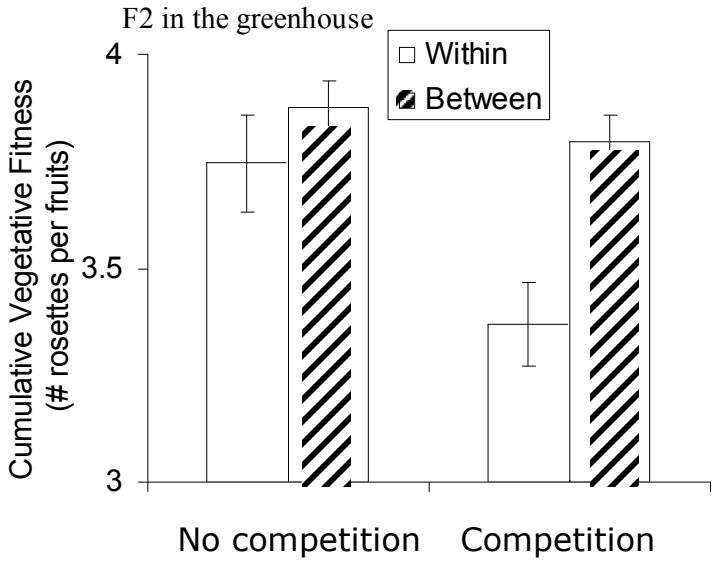


Figure 4: Means of residual vegetative fitness over all plants from each of the 13 natural populations of *Lychnis flos-cuculi* for offspring of F2 plants after within- and between-population crosses without or with competition. F2 vegetative fitness in the greenhouse with competition was marginally significantly higher in plants from between than within population crosses (Competition x Cross interaction, p<0.1). Levels of significance were obtained with hierarchical ANCOVA (Table 4).

▶▶

Table 3: Analyses of covariance for survival, vegetative and cumulative fitness of F1 transplanted to the natural populations after crosses within- and between-populations with plants from 13 natural populations of *Lychnis flos-cuculi*. Factors and covariates comprise a set of parental population characteristics, cross types, and genetic, geographic, and ecological distances between these populations. Population denotes the 13 parental populations. We square root transformed the data to meet the assumption for normality. We present mean squares (ms) or mean deviance changes (mdev), F and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001. Cross types are S:Small, L:Large, Si:Similar and Di:Different.

F1 fitness in the field	Vegetative				Reproductive				Survival			
Sources of variation	DF	ms	F		DF	ms	F		DF	ms	F	
Maternal Pop. A.R.	1	6.4	1.01		1	116.3	4.83	+	1	2.95	4.73	+
Maternal Pop size	1	72.1	11.27	*	1	17.5	0.73		1	0.02	0.04	
Maternal Pop. Humidity (F)	1	0.1	0.02		1	10.2	0.42		1	0.62	0.99	
Maternal Pop. Acidity (R)	1	0.0	0.01		1	16.2	0.67		1	3.17	5.07	+
Maternal Pop. Nutrient (N)	1	16.0	2.50		1	12.1	0.50		1	1.19	1.91	
Maternal Pop. Light (L)	1	4.6	0.72		1	127.4	5.30	+	1	0.97	1.55	
Maternal Population	6	6.4	0.98		6	24.1	0.83		5	0.62	3.17	*
Maternal plants	84	6.5	6.27	***	81	28.8	5.27	***	85	0.20	0.04	
Paternal Pop. A.R.	1	4.8	0.78		1	37.5	2.39		1	1.11	5.57	+
Paternal Pop Size	1	0.7	0.12		1	21.1	1.34		1	0.33	1.64	
Paternal Pop. Humidity (F)	1	0.2	0.04		1	0.2	0.01		1	1.28	6.42	*
Paternal Pop. Acidity (R)	1	4.5	0.72		1	95.7	6.08	*	1	0.02	0.09	
Paternal Pop. Nutrient (N)	1	1.8	0.29		1	0.0	0.00		1	1.58	7.94	*
Paternal Pop. Light (L)	1	7.8	1.26		1	26.0	1.65		1	1.68	8.46	*
Paternal Population	7	6.2	1.52		8	15.7	0.93		7	0.20	0.86	
Paternal plant	91	4.0	3.89	***	81	17.0	3.11	***	91	0.23	0.04	
Transplant Site	11	3.5	3.34	***	11	13.8	2.53	**	11	1.23	0.23	
Cross (W. vs B.)	1	14.8	4.10	+	1	38.5	8.53	***	1	0.30	1.28	
Cross Type (S, L, Si, Di)	3	2.0	0.56		3	0.9	0.20		3	0.08	0.36	
Geo. Dist	1	8.7	2.41		1	27.7	6.13	*	1	0.09	0.37	
Fst	1	5.7	1.58		1	11.8	2.62		1	0.01	0.06	
Mean Qst	1	0.4	0.11		1	0.7	0.15		1	0.06	0.25	
Humidity (F) Dist.	1	17.2	4.76	*	1	23.8	5.27	+	1	0.73	3.12	+
Acidity (R) Dist.	1	9.8	2.73		1	26.1	5.78	*	1	0.34	1.44	
Nutrient (N) Dist.	1	3.3	0.91		1	0.6	0.12		1	0.24	1.01	
Light (L) Dist.	1	4.8	1.33		1	15.0	3.33		1	0.19	0.83	
Cross x Maternal Pop. A.R.	1	0.1	0.02		1	4.8	1.07		1	0.05	0.20	
Cross Type x Maternal Pop. A.R.	3	0.6	0.16		3	5.1	1.12		3	0.11	0.49	
Geo. Dist x Maternal Pop. A.R.	1	6.5	1.81		1	74.7	16.55	**	1	0.12	0.53	
Fst x Maternal Pop. A.R.	1	0.0	0.01		1	1.5	0.33		1	0.01	0.02	
Cross Type x Paternal Pop. A.R.	3	1.0	0.27		3	17.9	3.97	+	3	0.15	0.65	
Geo. Dist x Paternal Pop. A.R.	1	0.8	0.21		1	4.3	0.96		1	0.30	1.27	
Fst x Paternal Pop. A.R.	1	4.5	1.23		1	0.7	0.15		1	0.19	0.82	
Maternal Pop x Paternal Pop	17	3.6	3.46	***	7	4.5	0.83		21	0.23	0.04	
Residual	471	1.0			166	5.5			716	5.47		

Backcrosses

Fitness after backcrosses after between-population crosses was not significantly different from fitness after within-population crosses. Again, the effect of between- versus within-cross and of the four cross types depended on the population for all traits. Plants from populations with lower allelic richness survived marginally significantly better when crosses were performed within populations (cross x maternal allelic richness: $F_{1,6}=5.68$, $p<0.1$).

Table 4: Analyses of covariance for survival, vegetative and cumulative fitness of F2 in the greenhouse without and with competition after crosses within- and between-populations with plants from 13 natural populations of *Lychnis flos-cuculi*. Effects comprise a set of parental population characteristics variables, and treatment effects. Population denotes the 13 parental populations. We root transformed the data to meet the assumption of normality. We present mean squares (ms) or mean deviance changes (mdev), F and p values : + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001. Cross types are S:Small, L:Large, Si:Similar and Di:Different.

F2 fitness	Vegetative				Reproductive				Survival			
Source	DF	ms	F	P	DF	ms	F	P	DF	ms	F	P
Maternal Pop. A.R.	1	0.11	0.03		1	2.55	1.05		1	0.14	0.44	
Maternal Pop size	1	2.76	0.72		1	3.98	1.64		1	0.97	2.99	
Maternal Population	9	3.83	1.36		10	2.43	0.53		9	0.32	1.37	
Maternal plants	82	2.81	8.44	***	78	4.55	3.71	***	86	0.24	5.55	***
Competition	1	9.35	58.27	+	1	78.18	232.58	*	1	0.00	0.00	
Cross (W. vs B.)	1	0.40	0.09		1	3.66	1.57		1	0.28	9.76	
Cross Type (S, L, Si, Di)	3	3.30	1.50		3	3.55	0.58		3	0.39	1.66	
Competition x Maternal Pop. A.R.	1	0.16	0.28		1	0.34	0.41		1	0.10	3.29	+
Cross x Maternal Pop. A.R.	1	4.67	4.64	+	1	2.33	1.40		1	0.03	0.22	
Cross Type x Maternal Pop. A.R.	3	2.20	0.62		3	6.09	1.33		3	0.23	1.10	
Competition x Maternal Pop.	10	0.57	1.71	+	10	0.83	0.68		10	0.03	0.74	
Cross x Maternal Pop.	7	1.01	3.02	**	7	1.67	1.36		8	0.13	3.08	**
Cross Type x Maternal Pop.	24	3.53	10.60	***	25	4.57	3.73	***	25	0.21	4.93	***
Competition x Cross	1	0.95	2.85	+	1	0.08	0.06		1	0.01	0.24	
Residual	441	0.33			292	1.23			495	0.04		

Between-population cross types

For the F1 in the greenhouse, offspring from crosses with larger populations had a lower vegetative fitness compared to the other cross types and offspring from crosses with an ecologically similar population had a higher vegetative fitness (cross type: $F_{1,22}=3.2$, $p<0.05$).

In contrast, the F1 transplanted to the field did not show any significant differences between the four cross types. Thus, the dichotomy within- versus between-population crosses appeared more important than the specific type of paternal population for offspring fitness under natural conditions.

For the F2, there were no significant main effects of the four cross types. Rather, the effects of the four cross types varied depending on the maternal population.

Table 5: Analyses of covariance for survival, vegetative and cumulative fitness of backcrosses in the greenhouse without and with competition after crosses within- and between-populations with plants from 13 natural populations of *Lychnis flos-cuculi*. Effects comprise a set of parental population characteristics variables, and treatments effect. Population denotes the 13 parental populations. We root transformed the data to meet the assumption of normality. We present mean squares (ms) or mean deviance changes (mdev), F and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001. Cross types are Small, Large, Similar and Different.

Ba fitness	Vegetative				Reproductive				Survival			
Source	DF	ms	F	P	DF	ms	F	P	DF	ms	F	P
Maternal Pop. A.R.	1	23.2	0.09		1	3.28	0.33		1	0.14	0.70	
Maternal Pop size	1	42.8	0.17		1	0.24	0.02		1	0.02	0.09	
Maternal Population	7	254.4	2.42	*	7	9.87	3.21	**	7	0.20	1.03	
Maternal plants	70	104.9	7.00	***	65	3.07	3.19	***	75	0.19	6.70	***
Competition	1	398.1	63.37	+	1	42.58	144.0	+	1	0.03	18.2	
Cross (W. vs B.)	1	343.5	4.32		1	4.72	0.63		1	0.00	0.00	
Cross Type (S, L, Si, Di)	3	50.1	10.55	*	3	5.89	3.01		3	0.26	1.35	
Competition x Maternal Pop. A.R.	1	6.3	0.75		1	0.30	0.41		1	0.00	0.19	
Cross x Maternal Pop. A.R.	1	79.6	1.46		1	7.48	1.74		1	0.73	5.68	+
Cross Type x Maternal Pop. A.R.	3	4.8	0.05		3	1.96	0.45		3	0.19	1.35	
Competition x Maternal Pop.	8	8.3	0.56		8	0.72	0.75		8	0.01	0.29	
Cross x Maternal Pop.	6	54.5	3.63	**	6	4.29	4.46	***	6	0.13	4.52	***
Cross Type x Maternal Pop.	13	99.7	6.65	***	10	4.34	4.52	***	14	0.14	5.06	***
Competition x Cross	1	4.3	0.29		1	0.00	0.00		1	0.00	0.04	
Residual	285	15.0			197	0.96			311	0.03		

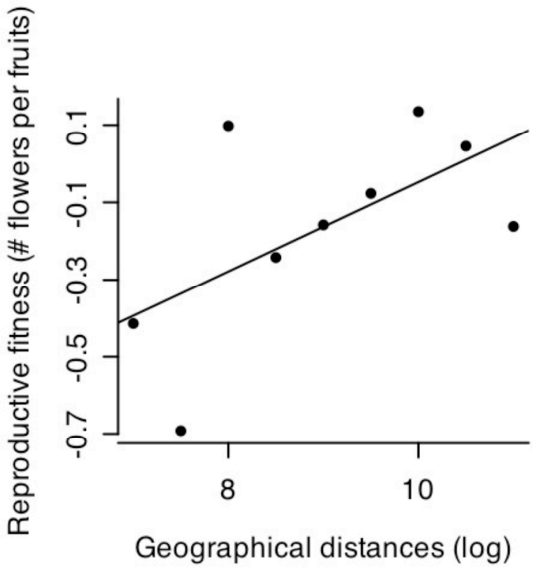
For backcrosses, the effects of the four cross types varied depending on maternal population and on the considered fitness trait. Crosses with ecologically more different populations produced offspring of lower vegetative fitness compared to the other cross types ($F_{1,3}$=10.6, $p<0.05$).

The effect of the type of cross on backcross performance and both generations of inter-population crosses changed depending on the population. Thus, the effect of fragmentation, as reflected in the genetic diversity of remnant populations did not interact with the type of cross in a consistent way.

Effects of geographic, genetic and ecological distances between populations

For the F1 in the greenhouse the effects of distances between populations were not significant. However, for the F1 transplanted to the field, plant reproductive fitness increased with larger geographical distance between parental populations ($F_{1,7}$=6.1, $p<0.05$) (Fig. 5), especially for plants from populations with higher allelic richness ($F_{1,7}$=16.55, $p<0.01$). Plant vegetative fitness increased with absolute difference in humidity indicator values between parental populations ($F_{1,17}$=4.8, $p<0.05$) and plant reproductive fitness increased with absolute difference in acidity indicator values between parental populations ($F_{1,7}$=5.8, $p<0.05$) (Fig. 6). To conclude, geographic and ecological distances between populations had an overall positive effect on plant performances after crosses between populations.

Figure 5: Means of residual reproductive fitness over all plants from each of the 13 natural populations of *Lychnis flos-cuculi* for F1 offspring transplanted to the field after within- and between-population crosses. The positive effect of larger geographic distances for reproductive fitness in the F1 plants transplanted to the field was significant ($p<0.05$).
Symbols represent means per population of origin and per transplant target sites, grouped into classes of geographical distances. Levels of significance were obtained with hierarchical ANCOVA (Table 3).



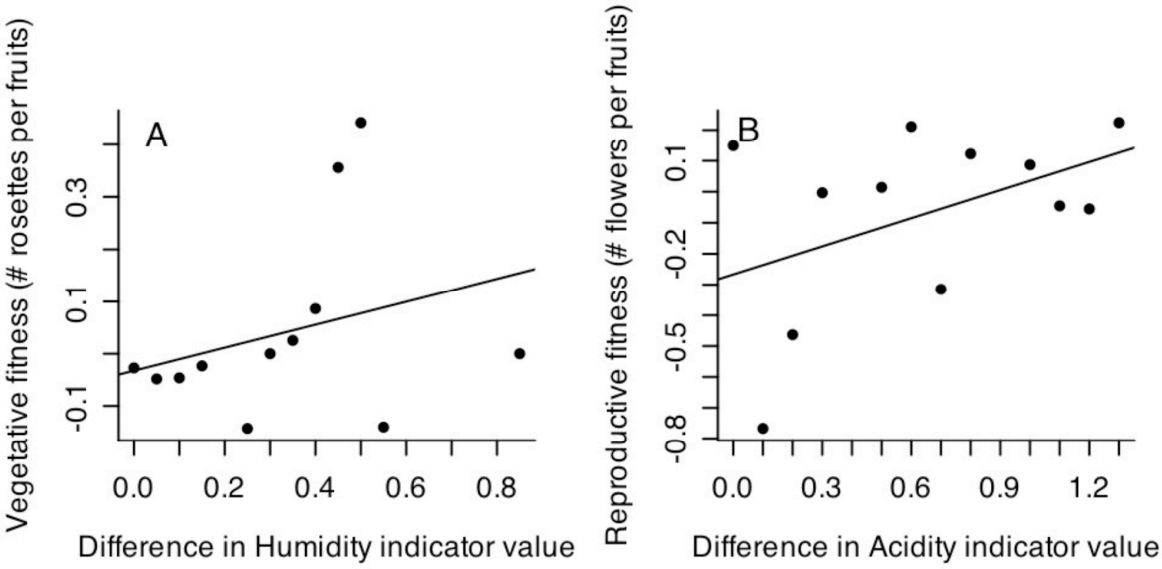


Figure 6: Means of residual fitness over all plants from each of the 13 maternal populations of *Lychnis flos-cuculi* for F1 offspring transplanted to the field after within- and between-population crosses. Effects of ecological distances in humidity were positive for vegetative fitness (A) ($p<0.05$) and in acidity for reproductive fitness (B) ($p<0.05$). Ecological distances were measured as the absolute differences in Landolt (1977) indicator value between the vegetation composition of parental populations. Symbols represent means per population of origin and per transplant target site, grouped into classes of absolute difference of ecological indicator value. Levels of significance were obtained with hierarchical ANCOVA (Table 2).

DISCUSSION

Genetic Allee effect

Offspring of maternal plants from large and genetically more diverse populations performed better than of plants from small populations. This extends earlier results (Galeuchet *et al.* 2005b) as it concerns offspring resulting from crosses with different populations and as it concerns offspring grown across a range of environmental conditions.

Genetic rescue

Genetic rescue can replenish genetic variation and reduce inbreeding in small populations. Therefore, it usually creates at first a generation of plants which are heterozygous for most loci, and thus deficient alleles are much more likely to be compensated by a normal allele (Frankham 1995). For our 13 populations of *L. flos-cuculi*, both vegetative and reproductive cumulative fitness were higher after between-population crosses in the greenhouse and in the field. Hauser had found for four populations of *L. flos-cuculi* a higher fitness in the offspring after between-population crosses compared to within-populations crosses (Hauser & Loeschcke 1994), and the same author showed that crosses between populations of *Silene nutans* produced zygotes more likely to survive than zygotes from within-population crosses (Hauser & Siegismund 2000). Similar effects were found for a whole range of species and traits including *Arnica montana* (Luijten *et al.* 2002), *Scorzonera humilis* (Colling *et al.* 2004) and *Silene alba* (Keller *et al.* 2000). The positive heterosis effect in *Silene littorea* was due to reversing inbreeding depression rather than increasing genetic diversity per se (Vilas *et al.* 2006). Even within populations, Dudash found fitness advantages for far-outcrosses compared to near-outcrosses with *Sabatia angularis*, probably because it reduced the strong inbreeding due to local population structure (Dudash 1990). Thus, heterosis after one generation of outbreeding seems to be the rule rather than the exception. However, the low number of

populations involved in these previous studies did not allow them to test effects of population characteristics or distances between populations on the consequences of between-population crosses.

Outbreeding depression

Most fitness traits in the F1 were non-significant and vegetative fitness of the F2 and survival of the backcrosses were marginally significantly higher for within-population crosses for populations with low allelic richness. This suggests tendencies for some outbreeding depression for plants of small, genetically depauperate populations. This could be due to purging of deleterious alleles in smaller populations. Indeed the fitness of between-populations crosses varied little across different population sizes, whereas the fitness of within-population crosses decreased significantly with maternal population size. On the other hand, it could be explained by the action of genetic drift on plant genomes. Mixing different gene pools could then break-up co-adapted gene complexes. In an experiment with *Papaver rhoeas,* biomass was negatively influenced by outbreeding caused by epistasis in all four generations, and seed mass was decreased in the F2 (Keller *et al.* 2000). Outbreeding depression was observed in *Gentianella germanica* after crosses between populations (Fischer & Matthies 1997). Waser and co-authors found that outbreeding effects vary for the same species in space and time (Waser *et al.* 2000). These examples illustrate why gene flow has been termed the Jekyll and Hyde of conservation (Stockwell *et al.* 2003).

No break-up of local adaptation

Plants transplanted to the field populations did not show a break-up of local adaptation, although a reciprocal replant-transplant experiment reported that *L. flos-cuculi* plants from our populations are adapted to ecological gradients (Chapter 3). Moreover, in the field plants showed a similar heterosis effect than in the greenhouse. A similar advantage for plants from between-populations crosses compared to within-population

crosses was found for *Scabiosa columbaria* between populations at distances of up to 160 km (Van Treuren *et al.* 1993), which corresponds to the geographical range of our study. However, other studies found a break-down of local adaptation, even at a small scale. Within the same population of *Impatiens capensis*, outbred plants were doing worse than the inbred plants when growing exactly on the same site than the maternal plants, while the opposite was true for offspring growing further away from the maternal plant (Schmitt & Gamble 1990). Although the importance of choosing the right source for introduction of plant material was stressed in conservation and the need declared to delineate seed transfer zones (Hufford & Mazer 2003), our experiment suggests that this might not always be important. Rather, adequate seed transfer zones might be quite large.

Effects of geographic, genetic and ecological distances between populations

Offspring fitness increased monotonically with increasing geographic and ecological distances between populations. Thus, we did not detect an optimum outbreeding distance (Waser 1993a; Willi & Van Buskirk 2005). Possibly, at even further crossing distances than the ones used in our study, maximum offspring fitness might have been reached.

This confirms that the break-up of local adaptation does not occur in *L. flos-cuculi* within this range of geographic and ecological distances. Thus, when introducing plant material from distant sources break-up of local adaptation might be less important than previously thought.

Conclusion

Our study with many populations allowed us to evaluate the effects of population characteristics, and of several types of inter-population distances for consequences of inter-population crosses. Overall, we conclude that heterosis outweighs potentially negative effects of inter-population crosses, at least in *L. flos-cuculi*.

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Chapter 6

Summary

Zusammenfassung

Résumé

Summary

Fragmentation of natural and semi-natural habitats is among the major threats to biodiversity. It can strongly decrease biodiversity at the species and at the genetic level. It impairs both rare and common species and can reduce overall plant performance in small populations. This reduction in individual fitness triggers a decrease in population fitness and risks of local extinction increase both at the population and at the species level. Plants can respond to changes in habitat quality via plasticity. Other can adapt to the local conditions, which, however, can reduce their capacity to adapt to new environmental conditions. Genetic erosion, via drift and inbreeding, can cause a fitness decrease, and may be compensated via genetic rescue. Outbreeding between populations has unfortunately showed positive, but also negative effects and it needs to be better understood before any restoration management can proceed with high chances of success.

Effects of habitat fragmentation can also be found at the community level, where biotic interactions can be disrupted. For example pollination varies strongly with population size, and therefore can change the effect and strength of pollen diversity.

I examined the interaction between habitat fragmentation, population genetics and local adaptation. I first asked what the consequences are of longer-term inbreeding for the fitness of *L. flos-cuculi* plants from a fragmented landscape. I tackled the interaction between habitat fragmentation and local adaptation, which had not before been simultaneously addressed, in a reciprocal replant-transplant experiment among 15 populations. I also looked at pollen diversity and its effects on plant performances and on population via pollen competition, which is an often neglected level of diversity, and there I tried to separate selection and complementarity effects with a pollen diversity experiment, for the first time applying a method from community ecology. To finish, I

focused on the effects of inter-population crosses for 13 populations of *L. flos-cuculi*, 6 small and 7 large.

My model species *Lychnis flos-cuculi* (Caryophyllaceae) is still common throughout all Europe. However, its habitat has decreased in the last decades due to agriculture and urbanisation, with up to 90 percent of wet meadows disappearing in Switzerland alone. Its populations have been declining at the same time, and therefore it is a good model for studying the process of habitat fragmentation.

Isolation of populations and small population size may increase rates of inbreeding within populations. Higher inbreeding rates in such populations may lower plant fitness via inbreeding depression (ID), if ID is the same in all populations. However, depending on population history ID may differ between populations and change over generations because of dominance and epistasis or because of selection purging deleterious mutations.

In the fragmented landscape, isolation of populations and small population size increase rates of inbreeding within populations. This may lower plant fitness via inbreeding depression. However, depending on population history inbreeding load may differ between populations. It can change over generations because of dominance and epistasis or because of selection against deleterious alleles. I tested in **Chapter 2** the consequences of three generations of crosses of different degrees of experimental selfing and outcrossing for plants of the self-compatible perennial plant species *Lychnis flos-cuculi* from 19 populations in North-East Switzerland. These populations of plant origin differed in size and genetic diversity. Inbreeding depression was expressed in all generations and at the stages of seed production, germination and adult plant size. Genetic load was substantial in small populations and it was not purged rapidly under selfing. While few interactions between levels of experimental inbreeding and genetic variation of the population of origin suggested purging of inbreeding load from genetically less variable natural

populations, others did not. I conclude that common plants, such as *Lychnis flos-cuculi,* do not rapidly purge their inbreeding load if populations get small and are therefore prone to negative genetics consequences of landscape fragmentation.

Fragmentation and changes in habitat quality can both affect plant performance but have not previously been simultaneously addressed. The relationship between habitat quality and plant performance may be ruled by plasticity or by local adaptation. I examined the relative importance of plastic responses to differences in habitat quality and local adaptation to ecological conditions for plant performance in the fragmented landscape (**Chapter 3**). I studied the common but declining perennial herb *Lychnis flos-cuculi* in fen grasslands in NE Switzerland in a reciprocal replant-transplant experiment among 15 populations during four years. At sites with high moisture and nutrient indicator values, transplanted *L. flos-cuculi* grew more rosettes thus indicating plastic responses. Plants originating from smaller populations grew fewer daughter rosettes and produced fewer flowering stems. This suggests that some populations of the common plant *L. flos-cuculi* have decreased to a size where they suffer from a genetically-based Allee effect. Plants originating from populations with a light indicator value different from the one at the transplantation site had reduced survival and growth at the transplantation site, suggesting adaptation to an ecological gradient. These results indicate that both habitat fragmentation, through reduced population size, and adaptation to ecological gradients contribute to the performance of *L. flos-cuculi* in modern landscapes. I conclude that habitats should be protected in adequate sizes even for common plant species. This study also call for caution in selecting adequate plant material in ecological restoration or compensation projects to avoid break-up of population differentiation and local adaptation to ecological gradients.

Multiple paternity is common in natural plant populations, enabling pollen interactions and possibly fitness effects of pollen diversity. This

should be especially important in small, fragmented and therefore genetically eroded populations. In **Chapter 4**, I report a greenhouse pollination experiment with two levels of pollen diversity (one and four pollen donors). I used microsatellite analyses to assess paternity after four-pollen donor crosses. I used plants from eight populations to test whether large and small populations differ in effective paternity after four-donor-crosses. Moreover, I partitioned the effect of pollen paternity on offspring fitness into selection and complementarity effect. I found multiple non-random paternity after four-pollen-donor crosses. Higher experimental pollen diversity increased offspring fitness. This was due to positive complementarity effects between pollen of different donors, while selection effects were small and negative. Both non-random paternity after four-pollen-donor crosses and positive pollen diversity effects on offspring fitness were higher for plants from small than from large populations. This suggests that pollen diversity plays an important role in the context of small, fragmented populations and may be a previously overlooked mechanism for small populations to slow down genetic erosion.

I evaluated the consequences of between-population crosses for plants of 13 populations of the common but declining *Lychnis flos-cuculi* (**Chapter 5**). Previous information on molecular diversity and ecological conditions allowed me to study the roles of genetic diversity, and of genetic and ecological, along with geographical, distances between populations for offspring fitness. We crossed 7 plants of each of the 13 populations with a plant from: 1/ the same population, 2/ a small, 3/ a large, 4/ an ecologically similar and 5/ an ecologically dissimilar population to obtain the F1 generation. I grew plants of this F1 generation until flowering both in the greenhouse and in the field populations of origin. Moreover, I used the greenhouse plants to perform F2 and backcross crosses. Offspring of crosses between populations had higher vegetative and reproductive cumulative fitness in the F1 both in the greenhouse and in the field, indicating heterosis rather than breaking-up

of local adaptation. F1 performance also increased with larger geographic and ecological distances between parental populations. Although the heterosis effect was still positive in the F2 and backcrosses, it was not significant any more. Within-population crosses tended to be more beneficial for populations with lower allelic richness for most fitness traits and generations. Nevertheless, overall our study suggests that heterosis after between population crosses outweighs potential negative consequences.

The effects addressed in these four separate studies might actually interact. Inbreeding could favour local adaptation at a very fine scale: mating with nearby relatives ensures that the genes selected for this very micro-habitat will be kept. However, this might be overruled by inbreeding depression. Pollen diversity could reduce inbreeding and inbreeding depression when non-self pollen is preferred but could at the same time reduce local adaptation if foreign pollen is more successful than local one. Local adaptation is impaired if crosses are performed between populations experiencing different ecological conditions. Pollen diversity either favours or deters pollen from other populations, thus strongly influencing the outcome of crosses between populations. Between populations crosses break the inbreeding effect at the first generation, but can also loose the advantages of purging, as it will introduce deleterious mutations that might have been purged previously. Thus, carefully studying these processes separately constitutes a first and necessary step to understand what happens in natural populations, and other exciting new questions for further experimenting and modelling.

CONCLUSION

In this thesis I had aimed to answer four new questions. First, inbreeding negatively affected plant performances of 19 natural populations and more and more along three inbreeding generations. Small populations showed some first signs of purging but this was slow and little in

comparison to the inbreeding depression they endure. Second, plants were adapted to local ecological conditions and this varied with the size of the populations, thus indicating an interference of habitat fragmentation with local adaptation processes that had not been shown before. Third, for the first time, I showed that pollen diversity was affecting offspring through complementarity effects rather than through selection, and that paternity was more skewed and effects on offspring fitness were larger in small compared to large populations, thus demonstrating a large influence of habitat fragmentation on pollen diversity effects. Fourth, crosses between populations increase efficiently genetic diversity, as the heterosis effect in the first generation showed, without breaking-down local adaptation. Effects were lost in the second generation and mother backcrosses.

This thesis demonstrates that habitat fragmentation had a negative impact on the model species *Lychnis flos-cuculi*. What should be stressed here is that this plant is a common, long-lived perennial and most of its habitat loss occurred in the second half of the 20th century. Therefore it is highly probable that I observed only the first signs of effects of habitat fragmentation and more negative effects will come.

Two of the strengths of this study were being part of a larger, 6 year study and using various approaches to tackle the issue of habitat fragmentation. This enables several recommendations to be made for the management of small threaten populations. First, as overall effects of small population size are highly negative, protected areas should be large enough and of sufficient quality to sustain larger populations. Second, genetic diversity should be made a priority and transfer of plant material should be considered, preferably coming from highly diverse populations. Third, effects of biotic interactions, such as pollen diversity in small populations cannot be neglected.

Concerning fundamental aspects, some open questions remain. Which conditions do favour purging of deleterious alleles in the fragmented landscape? The combined study of habitat fragmentation and

local adaptation has allowed new perspectives to be developed and more research is still needed on these complex interactions. But will local adaptation and/or habitat fragmentation prevent further populations' adaptation to their changing environment? Pollen diversity has proven to have effects on populations and plant fitness, and these effects varied depending on population size. What are the mechanisms underlying these complementarity and selection effects? Which species and population characteristics can predict to which extent outbreeding will be beneficial or detrimental for the short and long term future of the populations concerned? We need a combination of experimental approaches, longer term studies and meta-analysis on the growing body of literature on these subjects, and we should strongly promote the use of techniques from other disciplines.

To conclude, the study of habitat fragmentation is still a challenge, both for conservation and for fundamental research, but this challenge had to be taken on to ensure a brighter future for biodiversity.

Zusammenfassung

Die Fragmentierung von natürlichen und naturnahen Habitaten stellt heutzutage eine der grössten Bedrohungen von Biodiversität dar. Habitatfragmentierung verursacht gravierende Veränderungen der Landschaft und kann die Biodiversität bezüglich Artenvielfalt und genetischer Vielfalt stark verringern. Seltene und verbreitete Arten sind durch Habitatfragmentierung bedroht und die gesamte Leistungsfähigkeit von Pflanzen in kleinen Populationen verringert. Diese gesamte Fitness der einzelnen Individuen führt zu einer Abnahme der Fitness der Population und das Aussterberisiko erhöht sich auf Populations- und Artebene gleichermassen. Pflanzen können auf Änderungen in der Lebensraumqualität über Plastizität reagieren, andererseits kann Anpassung an den lokalen Zustand ihre Kapazität verringern, sich an neue Umgebungzustände anzupassen. Genetische Erosion über Drift und Inzucht verursacht eine Abnahme der Fitness und kann über genetische Rettung ausgeglichen werden. Outbreeding zwischen Populationen zeigte sowohl positive als auch negative Auswirkungen. Deshalb bedarf es weiterer Forschung, um gezielte Renaturierungsmassnahmen mit hohen Erfolgwahrscheinlichkeiten durchzuführen. Dieselben Effekte wurden auch auf der Ebene der Lebensgemeinschaften gefunden, in der biotische Wechselwirkungen gestört werden können. Bestäubung hängt zum Beispiel stark von der Populationsgröße und kann den Effekt und die Stärke der Pollendiversität folglich ändern.

Ich verwendete verschiedene Methoden, um die Interaktion zwischen Habitatfragmentierung und lokaler Anpassung zu überprüfen. Bisher wurde beides separat untersucht und ich integrierte auch das genetische Niveau. Ich untersuchte auch die Pollendiversität und ihre Effekte auf Leistungsfähigkeit der Pflanze und Population über Pollenkonkurrenz.

Meine Modellart *Lychnis flos-cuculi* (Caryophyllaceae) ist noch überall in Europa verbreitet. In den letzten Jahrzehnten wurde ihr

Lebensraum durch Intensivierung der Landwirtschaft und Verstädterung stark verringert. In der Schweiz beispielsweise verschwanden bis zu 90 Prozent Feuchtwiesen Gleichzeitig nahm die Anzahl an Populationen ab, so dass die Art ein gutes Modell darstellt, um den Prozess der Habitatfragmentierung zu studieren.

Isolierung von Populationen und kleine Populationsgrößen können Inzuchtrate innerhalb der Populationen erhöhen. Höhere Inzuchtraten in solchen Populationen können Pflanzenfitness über Inzuchtdepression senken, wenn Inzuchtdepression in allen Populationen gleich ist. Abhängig von der Populationsgeschichte kann sich Inzuchtdepression jedoch zwischen Populationen unterscheiden und über Generationen ändern sowohl durch Dominanz und Epistasis, als auch durch Selektion, die schädliche Mutationen bereinigt. In **Kapitel 2** untersuchten ich mit mein Kollegen die Konsequenzen von drei Generationen aus experimenteller Selbstbestäubung und von Kreuzungen innerhalb der Populationen mit Pflanzen der selbst-kompatiblen mehrjährigen Art *L. flos-cuculi* aus 19 natürlichen Populationen in der Nordostschweiz. Die Populationen der ursprünglichen Generation unterschieden sich in Größe und Isolierung, d.h. Entfernung voneinander. Inzuchtdepression (ID) war in allen Generationen und an den Stadien der Samen, der Sämlinge und der Pflanzen offensichtlich. ID wurde unterschiedlich ausgedrückt während der Stadien des Lebenszyklus und der Generationen. Außerdem zeigten Interaktionen zwischen den Niveaus der experimentellen Inzucht und genetischer Diversität der Ursprungspopulation an, dass die Inzucht etwas bereinigt wurde von den genetisch weniger variablen natürlichen Populationen. Diese Experiment zeigt, dass verbreitete Pflanzen wie *L. flos-cuculi* unter Inzuchtdepression leiden können, wenn genetische Diversität der Populationen wegen der Habitatfragmentierung kleiner wird. Außerdem zeigte meine Experiment, während das Bereinigen von Inzuchtdepression der genetisch weniger variablen Populationen offensichtlich war, dass Inzuchtdepression unter Selbstbestäubung nicht schnell bereinigt wird.

Änderungen in der Lebensraumqualität und -fragmentierung können Pflanzenfitness beeinflussen. Beides wurde bisher jedoch nicht gleichzeitig untersucht. Das Verhältnis zwischen Lebensraumqualität und Pflanzenleistung kann durch Plastizität oder durch lokale Anpassung gesteuert werden. Ich überprüfte den relativen Wert von plastischen Reaktionen zur Lebensraumqualität und -anpassung und von Effekten der Habitatfragmentierung für die Leistungsfähigkeit der Pflanzen in einer fragmentierten Landschaft (**Kapitel 3**). Dazu führte ich ein wechselseitiges Verpflanzungsexperiment unter 15 Populationen während 4 Jahren durch. An Orten mit hohen Feuchtigkeits- und Nährstoffindikatorwerten, erbrachte *L. flos-cuculi* mehr Rosetten, d.h. zeigte plastische Reaktionen. Die Pflanzen, die von kleinen Populationen abstammten, erbrachten weniger Rosetten und produzierten weniger Blühtriebe. Einige Populationen der häufigen Art *L. flos-cuculi* müssen sich also auf eine Größe verringert haben, in der sie unter einem genetischbegründeten Allee Effekt leiden. Die Pflanzen, die von Populationen mit einem zu dem am Transplantationsort unterschiedlichen Lichtindikatorwert abstammen, hatten Überleben und Wachstum am Transplantationsort verringert, was auf Anpassung an unterschiedliche Umweltbedingungen hindeutet. Unsere Resultate zeigen, dass Habitatfragmentierung durch verringerte Populationsgrößen und Anpassung an Umweltgradienten zu den genetischen Bestandteilen der Fitness von *L. flos-cuculi* in den modernen Landschaften beitragen. Ich schließe daraus, dass Lebensräume ausreichender Größen auch für verbreitete Pflanzenarten geschützt werden sollten. Vorsicht wird auch in der Auswahl geeigneten Pflanzenmaterials für ökologische Renaturierungsprojekte benötigt, um das Ende der Differenzierung von Populationen und lokale Anpassung an ökologische Gradienten zu vermeiden.

Biodiversitäteffekte werden meistens auf Artebene und der Ebene der genetischen Diversität untersucht. Andere Ebenen wie Pollendiversität werden häufig vernachlässigt. Jedoch ist in natürlichen

Pflanzenpopulationen mehrfache, nicht-zufällige Vaterschaft verbreitet, so dass vorteilhafte Bedingungen für Pollenkonkurrenz und mögliche Effekte der Diversität vorliegen. In **Kapitel 4** berichte ich über ein Gewächshausexperiment mit Pflanzen aus 8 Populationen unterschiedlicher Größen nach Kreuzungen mit zwei Diversitätsniveaus (1 und 4 Pollenspender) sowie Vaterschaftanalysen mit Microsatelliten. Außerdem verwendete ich eine neue Analysemethode, um den Effekt der Pollenvaterschaft auf Nachkommensfitness in Auswahl- und Komplementaritätseffekt aufzutrennen. Ich fand nicht-zufällige Vaterschaft und einen positiven Effekt von höherer Pollendiversität auf Nachkommensfitness, meistens wegen des positiven Komplementaritätseffektes zwischen Pollenspendern, während der Auswahleffektes niedrig und negativ war. Ich zeigte auch zum ersten Mal, dass nicht zufällige Vaterschaft und Vorteile für Nachkommensfitness von den mehrfach hervorgebrachten Früchten in kleinen Populationen höher waren im Vergleich zu großen. Pollenwettbewerb spielt eine unerwartet wichtige Rolle im Zusammenhang mit kleinen, fragmentierten Populationen und ist eine der letzten Mechanismen, damit kleine Populationen genetischen Zusammenbruch verlangsamen und möglicherweise der Aussterbung entgehen können.

Ich untersuchte die Effekte der lokalen Anpassung, der Differenzierung von Populationen und der Populationsgröße auf das Ergebnis der gemischten Genpool zwischen Populationen (**Kapitel 5**). Um die Konsequenz der Anpassung an die Heterogenität und Fragmentierung der Landschaft auszuwerten, kreuzte ich 7 Pflanzen von je 13 Populationen mit einer Pflanze von: 1/ der gleichen Population, 2/ einer kleinen, 3/ einer großen, 4/ einer ökologisch ähnlichen und 5/ einer ökologisch unähnlichen Population. Ich führte F2- und Rückkreuzung im folgenden Jahr durch. Wir wissen, dass unsere Populationen lokale Anpassung an den ökologischen Gradienten zeigen und dass Pflanzenfitness einen Allee Effekt erreicht, wenn man große und kleinen Populationen vergleicht. Kreuzungen zwischen Populationen ergaben eine

höhere vegetative und reproduktive kumulative Fitness in der F1 Generation im Gewächshaus und im Feld und zeigten hybride Stärke der ersten Auszuchtgeneration. Die Heterosiseffekte waren in der F2 Generation nicht bedeutend. Kreuzungen innerhalb der Population waren vorteilhafter für kleine im Vergleich zu großen Populationen. Falls ein Import von Pflanzenmaterial erforderlich ist, um das Niveau der genetischen Diversität der bedrohten Populationen zu erhöhen, empfehle ich dass, extreme Vorsicht, und nur Pflanzen und Samen von hochqualität Population zur Verfügung zu stellen.

Die Resultate dieser vier unterschiedlichen Studien bilden komplizierte Interaktionen, wenn sie zusammen genommen werden. In der Tat könnte Inzucht lokale Anpassung in einer sehr kleinen Skala bevorzugen: Paarung mit nahen Verwandten stellt sicher, dass die Gene, die für diesen Mikro-Lebensraum ausgewählt wurden, erhalten werden. Pollendiversität könnte die Inzucht und die Inzuchtdepression verringern, wenn Fremdpollen bevorzugt wird, aber könnte gleichzeitig lokale Anpassung reduzieren, wenn fremder Blütenstaub erfolgreicher als einheimischer ist. Lokale Anpassung wird auch verhindert, wenn Kreuzungen zwischen Populationen unterschiedlicher ökologischer Bedingungen durchgeführt werden. Pollendiversität bevorzugt entweder Pollen oder hält ihn von anderen Populationen ab und beeinflußt so stark das Resultat der Kreuzungen zwischen Populationen. Kreuzungen zwischen Populationen brechen den Inzuchteffekt der ersten Generation, aber können die Vorteile des Bereinigens auch verlieren, da es zu schädlichen Mutationen kommen könnte, die vorher bereinigt worden wären. Das sorgfältige separate Untersuchen dieser vielen Einflüsse ist nur ein erster und notwendiger Schritt, die Komplexität dessen, was in natürlichen Populationen geschieht, sollte nicht außer Acht gelassen werden und kann zum Beispiel durch Modellieren stufenweise angepackt werden.

Schlussfolgerungen

In dieser Arbeit zielte ich darauf ab, vier neue Fragen zu beantworten. Zuerst beeinflußte Inzucht negativ die Pflanzenfitness in 19 natürlichen Populationen und mehr und mehr entlang drei Inzuchtgenerationen. Kleine Populationen zeigten einige erste Zeichen des Bereinigens, aber dieses war langsam und wenig im Vergleich zur Inzuchtdepression, der sie standhalten. Zweitens passten sich Pflanzen abhängig von der Populationsgröße ihren lokalen ökologischen Umgebungen an. Dies weist auf einen starken Effekt der Habitatfragmentierung auf Prozesse der lokalen Anpassung angezeigt waren, die nicht vorher bekannt waren. Drittens konnte ich zum ersten Mal zeigen, dass Pollendiversität Nachkommen durch Komplementarität und nicht durch Selektion beeinflusste, dass Vaterschaft unregelmässig war und Effekte auf Nachkommensfitness in kleinen verglichen mit großen Populationen größer waren und so einen großen Einfluß der Habitatfragmentierung auf Pollendiversitätseffekte demonstrierten. Viertens erhöhen Kreuzungen zwischen Populationen leistungsfähig genetische Diversität, wie der Heterosiseffekt in der ersten Erzeugung zeigte. Diese Effekte gingen in der zweiten Generation und Mutterrückkreuzungen verloren.

Diese Arbeit hat gezeigt, dass Habitatfragmentierung eine negative Auswirkung auf die Modellart *Lychnis flos-cuculi* hatte. Besonders betont werden sollte, dass es sich um eine häufige, langlebige und mehrjährige Art handelt, die hohe Verluste ihres Lebensraumes in der zweiten Hälfte des 20. Jahrhunderts erlitt. Folglich ist es in hohem Grade wahrscheinlich, dass ich nur die ersten Zeichen von Effekten der Habitatfragmentierung beobachtete und mehrere negative Effekte folgen werden.

Das Besondere an dieser Studie war zum einen, dass sie Teil einer größeren Studie über sechs Jahre war, zum anderen, dass verschiedene Methoden verwendet wurden, um das Problem der Habitatfragmentierung zu untersuchen. Dies ermöglicht verschiedene Empfehlungen für das Management von kleinen bedrohten Populationen. Als erstes sollten, da

alle Effekte kleiner Populationsgrößen in hohem Grade negativ sind, geschützte Bereiche in der Lage sein, größere Populationen zu unterstützen. Zweitens sollte genetische Diversität prioritär behandelt werden und eine Übertragung von Pflanzenmaterial, das bevorzugt aus sowohl hochgradig unterschiedlichen als auch ökologisch ähnlichen Populationen stammt, sollte in Erwägung gezogen werden. Drittens sollte man biotische Interaktionen, z.B. Pollendiversität in kleinen Populationen, besonders berücksichtigen. Hinsichtlich der grundlegenden Aspekte bleiben einige offene Fragen. Welche Bedingungen könnten das Bereinigen schädlicher Allele in einer fragmentierten Landschaft bevorzugen?

Diese kombinierte Studie zu Habitatfragmentierung und lokaler Anpassung hat gezeigt, dass neue Perspektiven entwickelt wurden und gleichzeitig mehr Forschung notwendig ist in Bezug auf komplizierte Interaktionen. Werden aber lokale Anpassung und/oder Habitatfragmentierung eine weitere Anpassung der Populationen an ihre sich ändernde Umgebung verhindern? Es hat sich gezeigt, dass Pollendiversität Populationen und Pflanzenfitness beeinflusst und dass diese Effekte abhängig von der Größe der Populationen sind. Was sind die Mechanismen, die diesen Komplementaritäts- und Selektionseffekten zugrunde liegen? Welche Eigenschaften von Arten und Populationen können voraussagen, in welchem Ausmaß Outbreeding/Auszucht kurz- oder langfristig für die jeweiligen Populationen vorteilhaft oder schädlich ist?

Wir benötigen eine Kombination aus experimentellen Methoden, längerfristigen Untersuchungen und Metaanalyse der beständig wachsenden Literatur zu diesen Themen, und wir sollten den Gebrauch von Techniken anderer Disziplinen stark fördern.

Abschließend bleibt festzustellen, dass die Untersuchung von Habitatfragmentierung eine Herausforderung bleibt, sowohl für die den Naturschutz, als auch für die Grundlagenforschung, aber diese

Herausforderung sollte in Angriff genommen werden, um eine bessere Zukunft für die Biodiversität sicherzustellen.

Résumé

La fragmentation du paysage, et par conséquent des habitats naturels, est aujourd'hui l'une des plus grandes menaces qui pèse sur la biodiversité. Elle entraîne d'importants changements au niveau du paysage et peut fortement réduire la diversité des espèces animales et végétales, ainsi que la diversité génétique au sein de ces mêmes espèces. Elle diminue les performances des petites populations et menace à la fois les espèces rares ou fréquentes. La diminution des performances de l'ensemble des individus d'une population déclenche une diminution des performances de la population elle-même et les risques d'extinction augmentent pour les populations et donc pour l'espèce observée. Les plantes sont capables de supporter un changement de qualité de leur habitat grâce à la plasticité phénotypique ou une adaptation aux nouvelles conditions, mais une adaptation locale peut réduire leur capacité d'adaptation à de nouvelles conditions écologiques globales. L'érosion génétique, liée à la consanguinité et à la dérive génétique, diminue les performances des plantes par une réduction de leur qualité génétique mais peut être compensée par un apport de nouveaux allèles. Suivant cette idée, des croisements entre populations ont déjà été effectués et ont montré des effets positifs mais ils ont également montré quelques effets négatifs. Il est alors nécessaire de mieux comprendre les mécanismes produisant ces effets négatifs avant de tenter d'appliquer avec succès des mesures de renforcement des populations menacées. Ces différents effets peuvent se produire au niveau de la communauté, perturbant les interactions entre espèces. Par exemple, la pollinisation peut varier en fonction de la taille des populations et par conséquent peut changer les effets de la diversité pollinique.

Dans ce travail de thèse, j'ai appliqué différentes approches afin d'étudier spécifiquement les interactions possibles entre fragmentation de l'habitat, adaptation locale et génétique des populations. J'ai également

étudié la diversité pollinique et ses effets sur les performances des plantes et des populations.

L'espèce considérée ici, et utilisée comme modèle, est *Lychnis flos-cuculi* (Caryophyllaceae), une plante encore considérée comme fréquente dans toute l'Europe. Cependant, dû au développement de l'agriculture et à l'expansion de l'urbanisation, l'habitat naturel de cette espèce a fortement diminué ces dernières années. Ainsi, près de 90% des prairies humides de Suisse ont disparu. Le nombre et la taille des populations de *L. flos-cuculi* ont diminué durant la même période ce qui fait de cette espèce un bon modèle pour l'étude du processus de fragmentation de l'habitat.

Une isolation importante de la population et une taille réduite peuvent augmenter le taux de consanguinité à l'intérieur de la population. Un taux important de consanguinité diminue les performances des plantes par un phénomène nommé dépression de consanguinité. Cependant, cette dépression de consanguinité peut varier en fonction de l'histoire des populations et changer au cours des générations par des mécanismes comme la dominance et l'épistasie ou à cause de la purge sélective des mutations délétères. Afin de mettre en évidence ces différents mécanismes au niveau génétique, j'ai examiné avec mes collègues dans le **Chapitre 2** les conséquences de trois générations d'autopollinisation et d'allopollinisation (croisements entre plantes différentes mais appartenant à la même population) chez *L. flos-cuculi,* une plante pérenne auto-compatible. Les plantes utilisées proviennent de 19 populations naturelles de *L. flos-cuculi* situées dans le nord-est de la Suisse, populations qui ont à la fois des tailles et des degrés d'isolation différents. Une dépression de consanguinité est visible dans mes résultats, et cela pour toutes les générations et tous les stades de croissance des plantes (graine, plantule et plante adulte), mais est exprimée différemment en fonction de la génération et du stade de la plante dans son cycle de vie. De plus, l'interaction entre les niveaux de consanguinité expérimentaux et la quantité de variation génétique de la population d'origine indique une

légère purge de la dépression de consanguinité pour les populations naturelles présentant une variation génétique plus faible. Mon étude montre qu'une espèce fréquente comme *L. flos-cuculi* peut aussi subir une dépression de consanguinité si la fragmentation du paysage a engendré une diminution de la diversité génétique à l'intérieur des populations. De plus, bien qu'il y ait quelques indications de purge d'allèles délétères dans les populations les moins variables génétiquement, mon expérience a montré que la dépression de consanguinité n'est pas purgée rapidement en cas d'autopollinisation.

Les changements de qualité de l'habitat et sa fragmentation peuvent tous deux influencer les performances des plantes mais n'ont pas, jusqu'à présent, été étudiés simultanément. La relation entre la qualité de l'habitat et les performances des plantes peut dépendre de la plasticité phénotypique ou de l'adaptation locale des individus. J'ai étudié l'importance relative de la réponse plastique à la qualité de l'habitat et à l'adaptation locale, en relation avec les effets de la fragmentation de l'habitat, sur les performances des plantes (**Chapitre 3**). J'ai effectué une expérience de « transplantations réciproques » entre 15 populations durant 4 ans. Les plantules de *L. flos-cuculi* transplantés dans des sites présentant des valeurs élevées concernant les indicateurs d'humidité et de nutriment ont produit un plus grand nombre de rosettes, indiquant une réponse plastique des plantes à leur nouvel environnement. Les plantes provenant de petites populations ont produit moins de rosettes et moins de tiges florales que les plantes provenant de grandes populations. Cela indique que certaines populations de l'espèce *L. flos-cuculi* ont été suffisamment réduites en taille pour souffrir d'un effet Allee établit au niveau génétique. Les plantes provenant de populations dont la valeur de l'indice de luminosité est différente de celle du site de transplantation ont montré une croissance et une survie réduite dans ce même site, ce qui suggère une adaptation au gradient écologique de leur habitat. Mes résultats indiquent que la fragmentation de l'habitat, par la réduction de la taille des populations et l'adaptation aux conditions écologiques, agit

sur la part génétique influençant les performances de *L. flos-cuculi* dans le paysage d'aujourd'hui. Je conclus que des habitats de taille adéquate doivent être protégés même pour les espèces fréquentes. Cependant, il faut être prudent dans le choix du matériel végétal utilisé en cas de mesures de restauration afin d'éviter de supprimer l'adaptation et la différenciation des populations liées aux gradients écologiques locaux.

Les recherches sur la biodiversité sont principalement concentrées au niveau des espèces et à la diversité génétique de ces dernières. La diversité pollinique en revanche a été négligée alors que la paternité d'un fruit dans les populations naturelles de plantes est souvent multiple et non aléatoire, engendrant ainsi des conditions favorables pour le développement de la compétition pollinique et de l'expression d'effets de diversité pollinique. Le **Chapitre 4** rapporte les résultats d'une expérience de pollinisation que j'ai menée en serre sur des plantes de 8 populations naturelles de différentes tailles. J'ai effectué des croisements en fonction de deux niveaux de diversité (1 ou 4 donneurs de pollen) et j'ai déterminé la paternité à l'aide d'une analyse microsatellite. De plus, lors du traitement des données, j'ai utilisé une nouvelle méthode qui permet de séparer les effets de la paternité sur les performances des descendants en deux composantes qui sont les effets de sélection et les effets de complémentarité. Selon les résultats, la paternité n'est pas aléatoire et l'effet d'une plus grande diversité de pollen est positif sur les performances des descendants. Cela est clairement lié à un effet de complémentarité entre les donneurs de pollen plutôt qu'à des effets de sélection qui sont faibles et négatifs. J'ai pu ainsi démontrer, également pour la première fois, que le biais dans la paternité et l'augmentation des performances des descendants lors de croisements avec plusieurs donneurs de pollen est plus fort dans les petites populations que dans les grandes. La diversité pollinique joue donc un rôle important pour les petites populations fragmentées et est l'un des derniers mécanismes qui permet aux petites populations de réduire l'érosion génétique.

J'ai étudié les effets de l'adaptation locale, de la différenciation entre les populations et de la taille des populations en effectuant des croisements entre populations (**Chapitre 5**). Pour estimer les conséquences de l'adaptation à l'hétérogénéité du paysage et à la fragmentation j'ai croisé 7 plantes de chacune des 13 populations avec une plante de : 1/ la même population, 2/ une petite population, 3/ une grande population, 4/ une population écologiquement similaire et 5/ une population écologiquement différente (première génération F1). L'année suivante, j'ai également produit une seconde génération de croisements (F2) et j'ai finalement effectué des croisements entre la première génération et la génération parentale. Les populations utilisées sont localement adaptées aux gradients écologiques de leur milieu et les performances des plantes présentent un effet Allee lorsque l'on compare les grandes et les petites populations. Les descendants F1 de croisements entre populations ont une meilleure fitness cumulative pour la reproduction et la croissance dans la serre et une fois transplantés dans leurs populations d'origine, indiquant une vigueur hybride pour la première génération de croisements entre populations. En fonction de ces résultats, je conseille de porter une grande attention au choix des plantes pour accroître la diversité génétique de populations menacées.

Les résultats de ces quatre études, examinés ensemble, forment un système complet et complexe. En effet, la consanguinité peut favoriser l'adaptation locale à très petite échelle : les croisements entre proches voisins garantissent que les gènes favorables et sélectionnés pour le micro habitat où ils se trouvent seront préservés. Cependant, simultanément la consanguinité est également défavorable en permettant l'expression plus fréquente de gènes délétères réduisant les performances des plantes. La diversité pollinique peut réduire la consanguinité et la dépression de consanguinité si le pollen génétiquement le plus différent de la plante mère est préféré mais simultanément cela pourrait aussi réduire l'adaptation locale. Cette dernière est aussi perturbée si des croisements sont effectués entre populations présentant des conditions

écologiques différentes. Il en résulte que la diversité pollinique peut favoriser ou défavoriser le pollen provenant d'autres populations et ainsi peut largement influencer le résultat de croisements entre populations. Les croisements entre populations quand à eux peuvent, d'une part, réduire fortement le taux de consanguinité en une génération, mais peuvent d'autre part réintroduire des mutations génétiques délétères qui avaient été éliminées par la consanguinité. Par conséquent, l'étude minutieuse de chacun de ces nombreux mécanismes n'est que la première, mais indispensable, étape à la compréhension de ce qui se passe dans les populations naturelles et d'autres approches telles que la modélisation seront nécessaires pour intégrer les nombreux facteurs impliqués.

CONCLUSION

Dans ce travail de thèse, mon but était de répondre à quatre nouvelles questions concernant les problèmes de fragmentation du paysage. Premièrement, je peux affirmer que la consanguinité affecte négativement les performances des individus de 19 populations naturelles non seulement dans leurs différents stades de croissance mais aussi au cours des générations. Dans les petites populations j'ai pu détecter les premiers signes de purge génétique mais cette purge est lente et faible en comparaison de la dépression de consanguinité subie par les individus. Deuxièmement, il apparaît clairement que les plantes sont adaptées à leurs conditions écologiques locales et cela en fonction de la taille des populations, indiquant ainsi un fort impact de la fragmentation de leur habitat sur le processus d'adaptation locale. Un tel résultat n'avait jamais été démontré jusqu'à présent. Troisièmement, j'ai pu montrer pour la première fois également que la diversité pollinique affecte les performances des descendants par un effet de complémentarité et pas par un effet de sélection. De plus, la paternité est plus biaisée et les effets sur les performances des descendants sont plus importants dans

les petites populations que dans les grandes, démontrant ainsi une grande influence de la fragmentation sur la diversité pollinique. Quatrièmement, par la vigueur hybride observée lors des croisements, je peux affirmer que les croisements entre populations augmentent bien la diversité génétique, sans perturber l'adaptation aux conditions locales dans le cas de *L. flos-cuculi*.

Cette thèse permet de démontrer que la fragmentation du paysage, et par conséquents des habitats naturels, a un impact fortement négatif sur l'espèce étudiée, soit *Lychnis flos-cuculi*. Il faut cependant insister sur le fait qu'il s'agit d'une plante pérenne fréquente et que la plus grande réduction de son habitat a eu lieu pendant la deuxième moitié du 20ème siècle. Il est par conséquent hautement probable que je n'ai observé que les tout premiers effets dus la fragmentation et que d'autres effets négatifs sont à attendre.

Les deux points forts de cette étude sont d'une part de faire partie d'un projet plus long, soit 6 ans, et d'autre part l'étude des problèmes liés à la fragmentation par différentes méthodes complémentaires. Les résultats permettent d'émettre plusieurs recommandations concernant la conservation de petites populations menacées. Tout d'abord, comme les effets d'une petite taille de population sont hautement négatifs, les surfaces protégées doivent être suffisamment grandes pour contenir de grandes populations. Ensuite, la diversité génétique devrait être une priorité et le renforcement en taille des petites populations avec un apport de plantes et de graines devrait être envisagé mais en utilisant de préférence des plantes provenant de populations génétiquement riches. Enfin, il faut être particulièrement vigilant aux interactions biotiques telles que la pollinisation et son influence sur la diversité pollinique.

Finalement, concernant les aspects de recherche fondamentale de nombreuses questions restent ouvertes. Quelles conditions favorisent la purge d'allèles délétères dans un paysage fragmenté ? L'étude simultanée de la fragmentation des habitats naturels et de l'adaptation locale a permis de développer de nouvelles perspectives et il est nécessaire

d'envisager d'autres études concernant ces interactions complexes. Est-ce que l'adaptation locale et-ou la fragmentation peuvent empêcher les plantes de s'adapter aux futurs changements de leur environnement ? La diversité pollinique semble capable d'influencer les performances des plantes et des populations, et ce en fonction de la taille des populations. Quels sont les mécanismes pouvant expliquer les effets de complémentarité et de sélection ? Quelles caractéristiques des populations et des espèces peuvent prédire la qualité des croisements entre populations, s'ils seront avantageux ou désavantageux à court terme et/ou à long terme ? Il me semble nécessaire pour répondre à ces différentes questions d'avoir une combinaison de différentes approches expérimentales, d'études à long terme et le développement de méta-analyses ainsi que de promouvoir l'utilisation de nouvelles techniques venant d'autres disciplines.

Pour conclure, j'aimerais souligner que l'étude de la fragmentation du paysage et des habitats naturels reste un grand défi, aussi bien pour la protection de la nature que pour la recherche fondamentale, mais ce défi doit être relevé pour assurer un avenir moins sombre à la biodiversité.

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